

ROLE OF MOESIN ABNORMALITIES IN NK/T CELL LYMPHOMAGENESIS

FUNCTIONAL ANALYSIS & IMPLICATION IN TUMOR IMMUNE ESCAPE

Amira MAROUF, MD, PhD Student

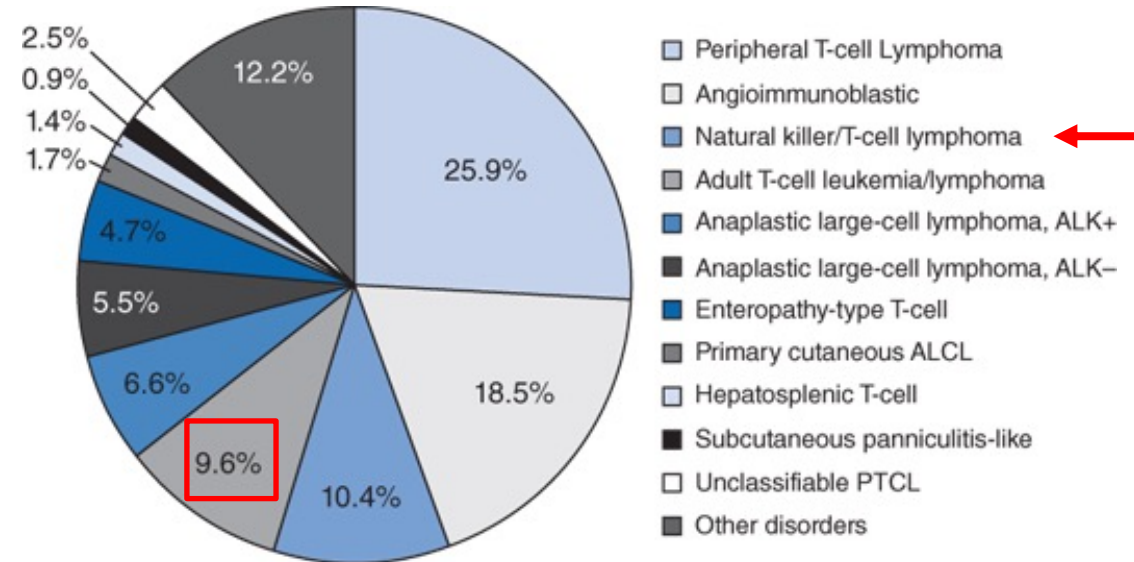
Supervisor: Dr Lucile COURONNÉ

« Laboratory of molecular mechanisms of hematological disorders and therapeutic implications »

Director: Pr Olivier Hermine

GENERALITIES ON NK/T LYMPHOMA (ENKTCL)

- Rare subset of peripheral **T/NK lymphoma** (10 %)
- Extranodal localization, most common presentation: **ORL involvement**
- **COO**: NK lymphocytes (40%) or T lymphocytes (60%)
- cCD3+, CD2+, CD5-, CD7+, CD56+, CD4-, CD8-
- Markers of cytotoxicity: Perforin+, GzmB+
- **EBV positive (EBER +) in 100% cases**

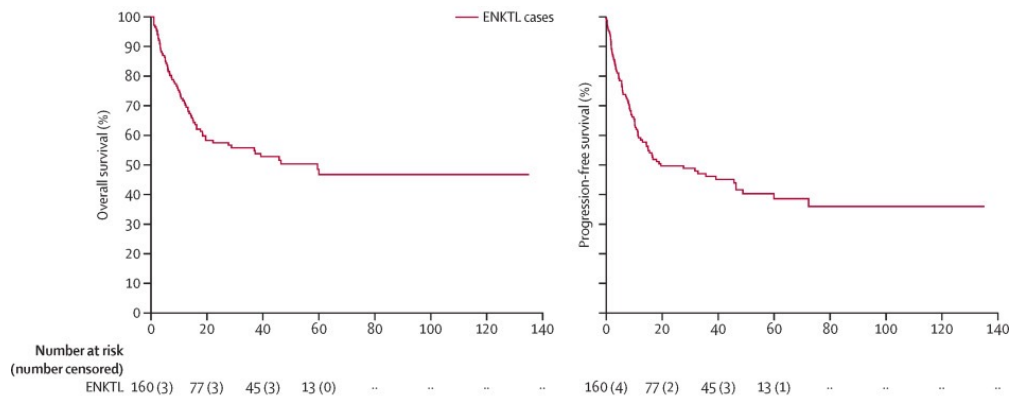


Source: Bruce A. Chabner, Dan L. Longo: Harrison's Manual of Oncology, 2nd Edition, www.hemonc.mhmedical.com Copyright © McGraw-Hill Education. All rights reserved.

GENERALITIES ON NK/T LYMPHOMA (ENKTCL)

- Associated with **poor prognosis**

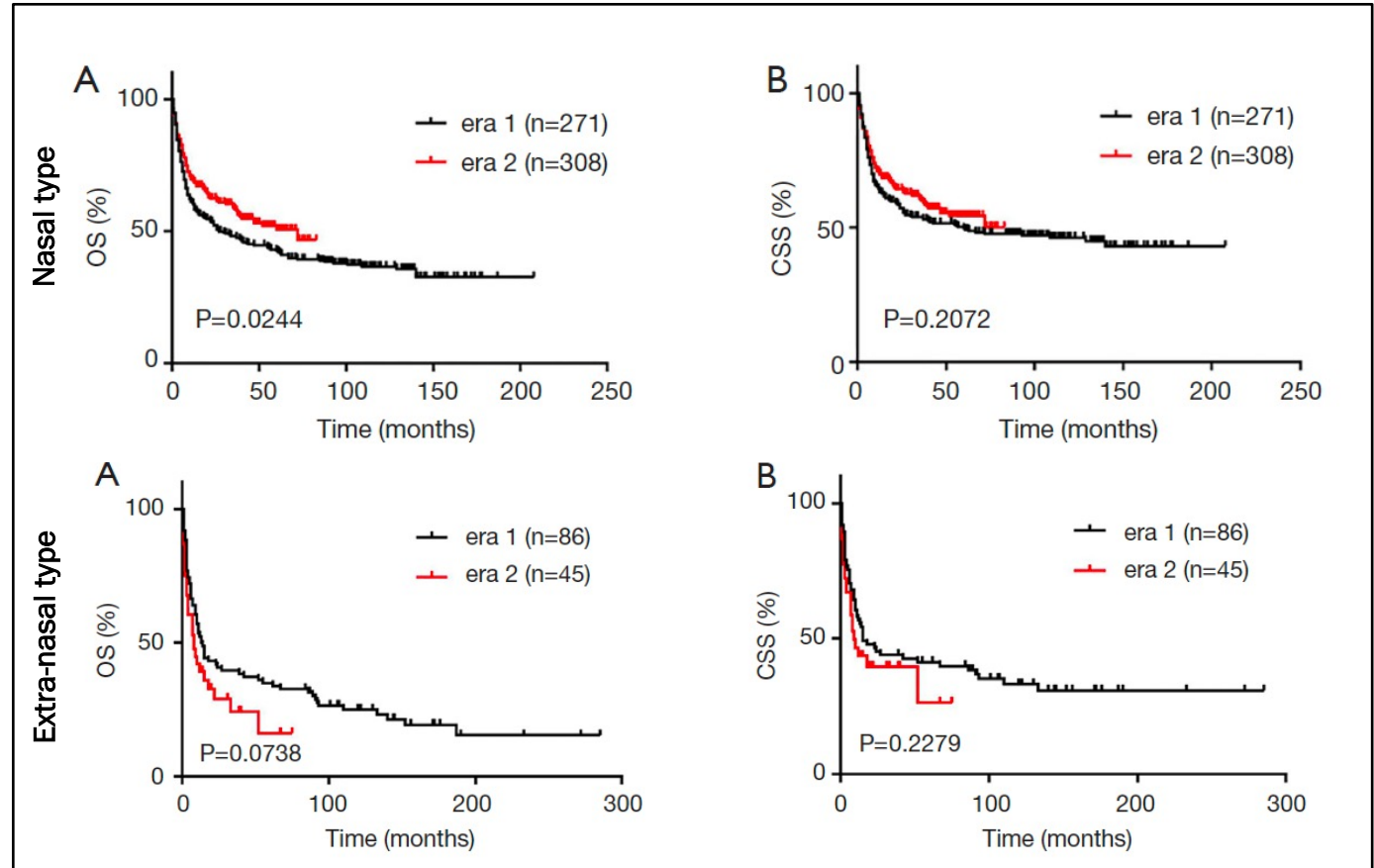
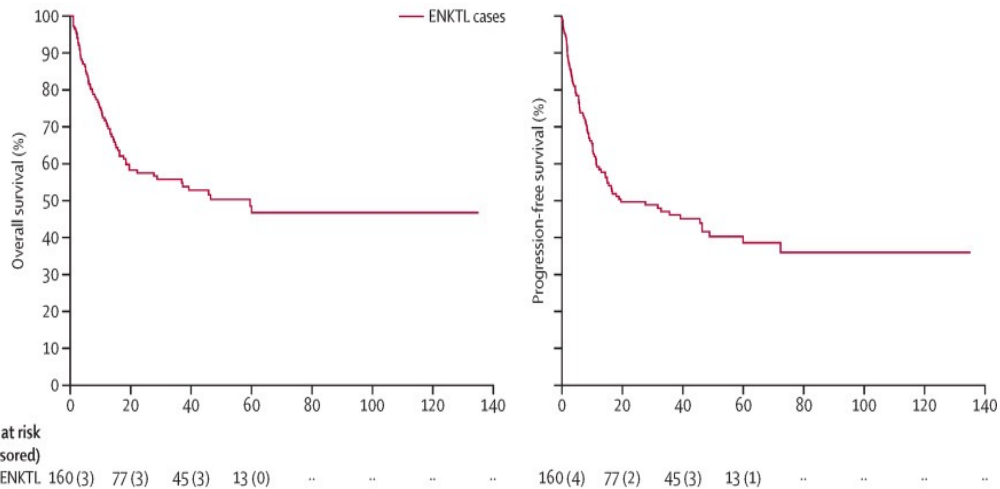
- Median OS 36 months
- Median PFS 57 months



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- Median PFS 57 months



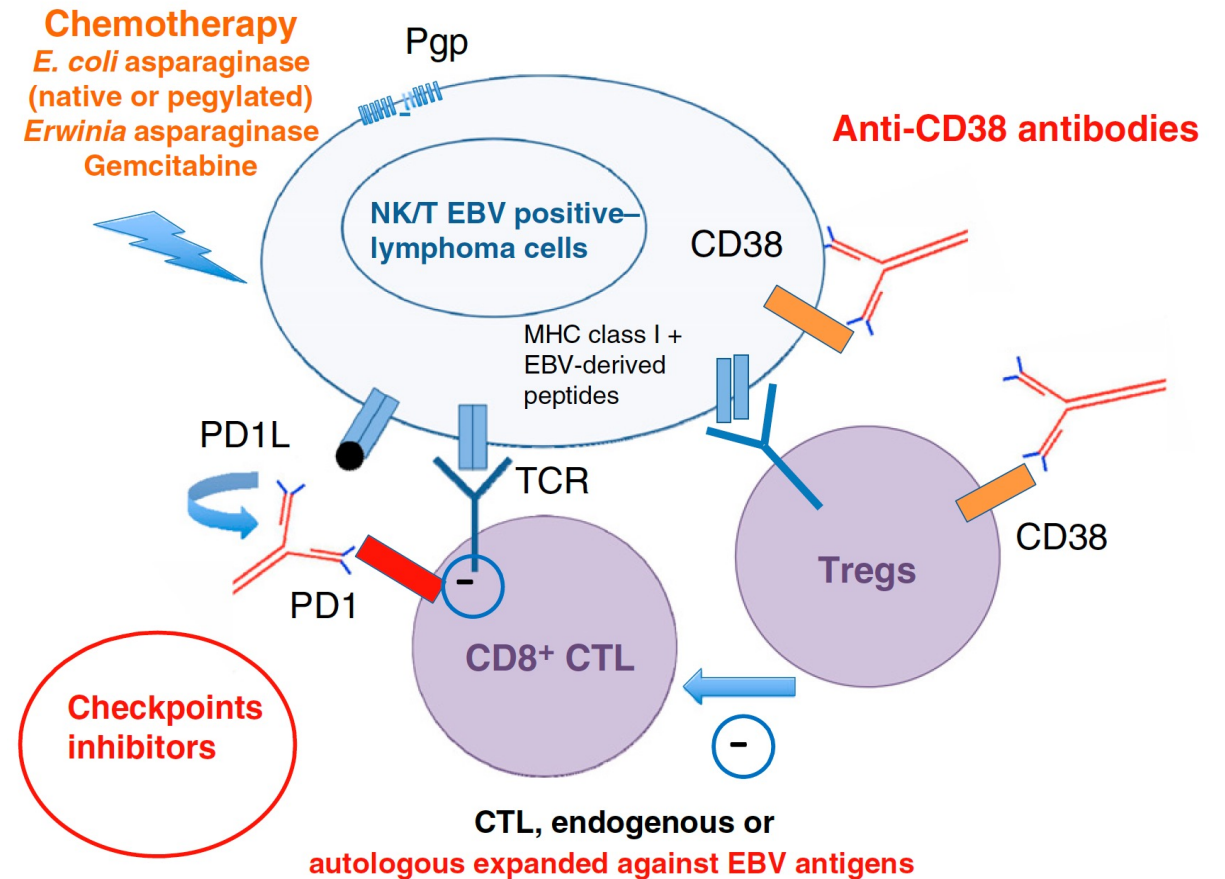
GENERALITIES ON NK/T LYMPHOMA (ENKTCL)

- Associated with **poor prognosis**
- New targeted therapies & **Immunotherapy** under clinical evaluation
 - Anti-CD30 antibodies
 - Anti-CD38 antibodies
 - HDAC inhibitors
 - **Checkpoint inhibitors: anti-PD1**
 - CTL anti-EBV

Table 5. Checkpoint-inhibitors in NKTL.

N Subjects		Response	Ref.
7	Pembrolizumab	5 CR/2 PR	[98]
7		2 CR/2 PR	[99]
14		5 CR/1 PR	[100]
3	Nivolumab	1 CR	[101]
28	Sintilimab	4 CR/15 PR	[103]
37	Sintilimab/Chidamide	16 CR/5 PR	[116]
6	Sintilimab/Chemotherapy	2 CR/4 PR	[102]
29	CS-001	7 CR/2 PR	[104]
21	Avelumab	5 CR/3 PR	[105]

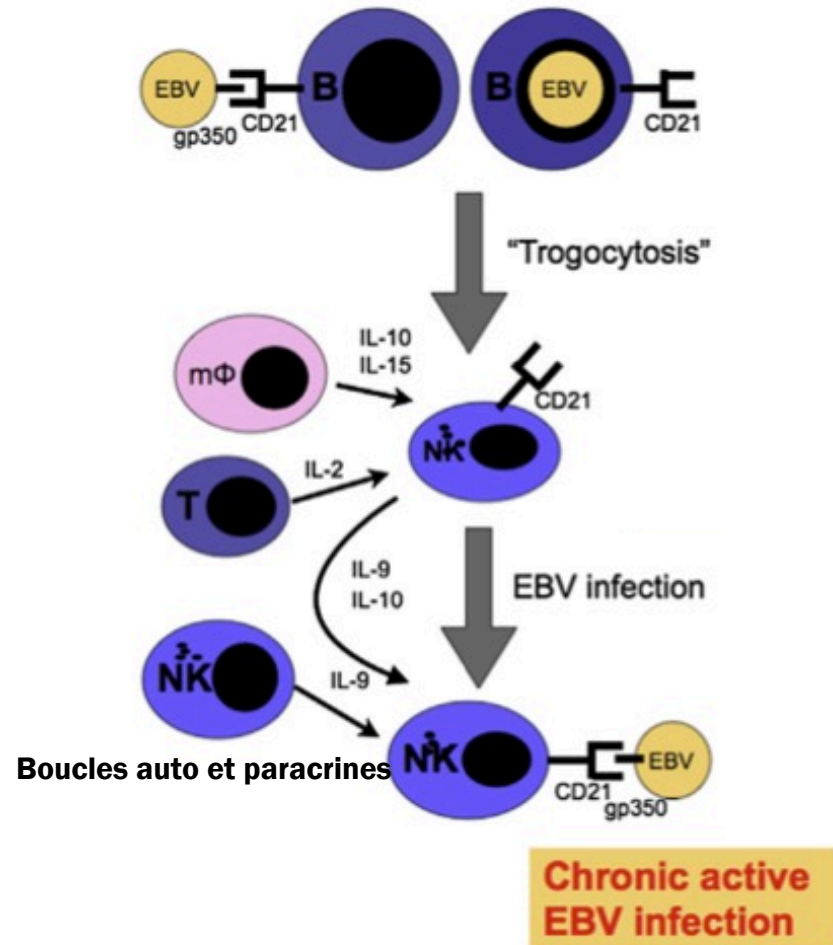
CR complete remission, PR partial remission.



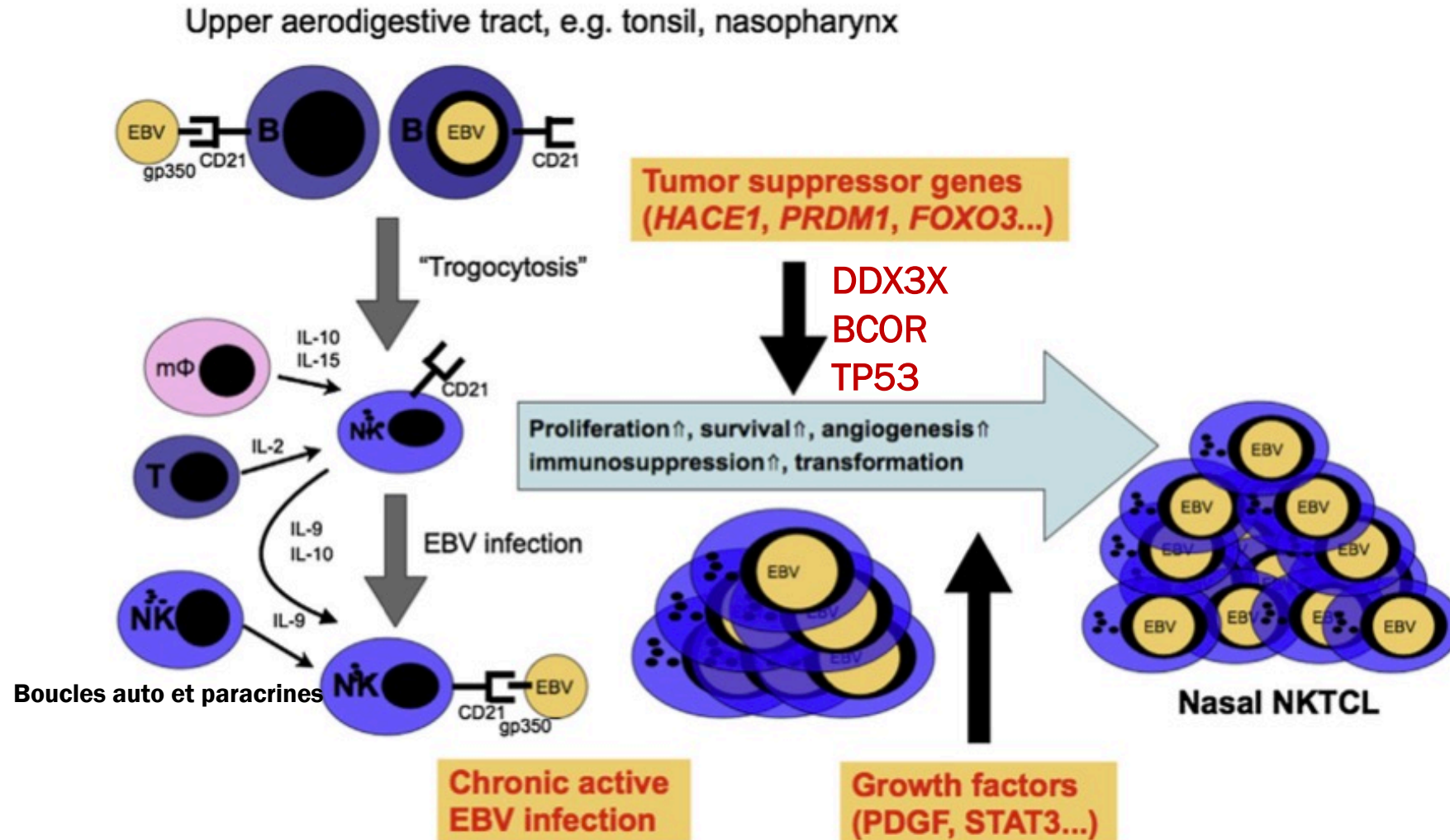
Jaccard & Hermine, *Blood*, 2017
 Fox, *Lancet Haematology*, 2020
 Qi, *Blood advances*, 2020
 He, *Ann. Of Translational Med.*, 2021
 Wang, *Leukemia*, 2021

PATHOGENESIS

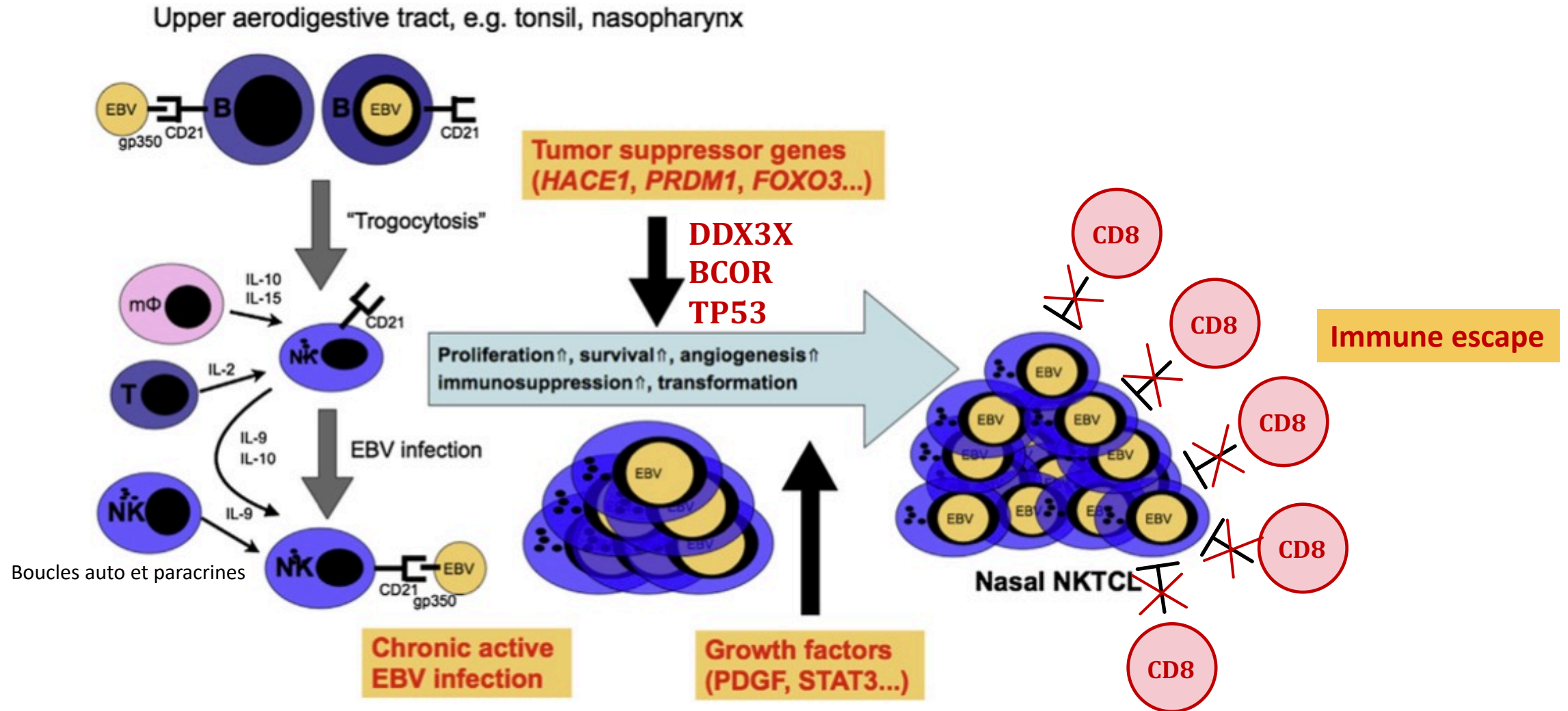
Upper aerodigestive tract, e.g. tonsil, nasopharynx



PATHOGENESIS

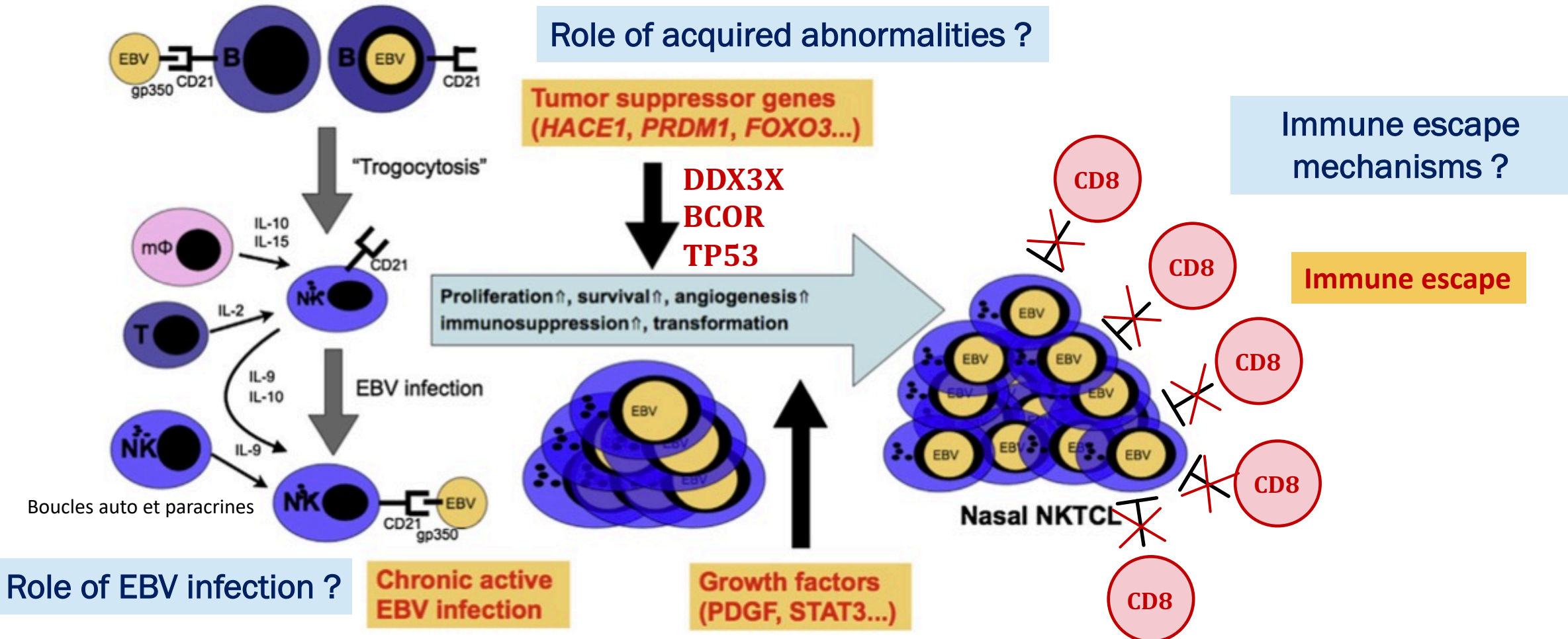


PATHOGENESIS



PATHOGENESIS

Upper aerodigestive tract, e.g. tonsil, nasopharynx



IDENTIFICATION OF MOLECULAR ABNORMALITIES INVOLVED IN ENKTCL LYMPHOMAGENESIS

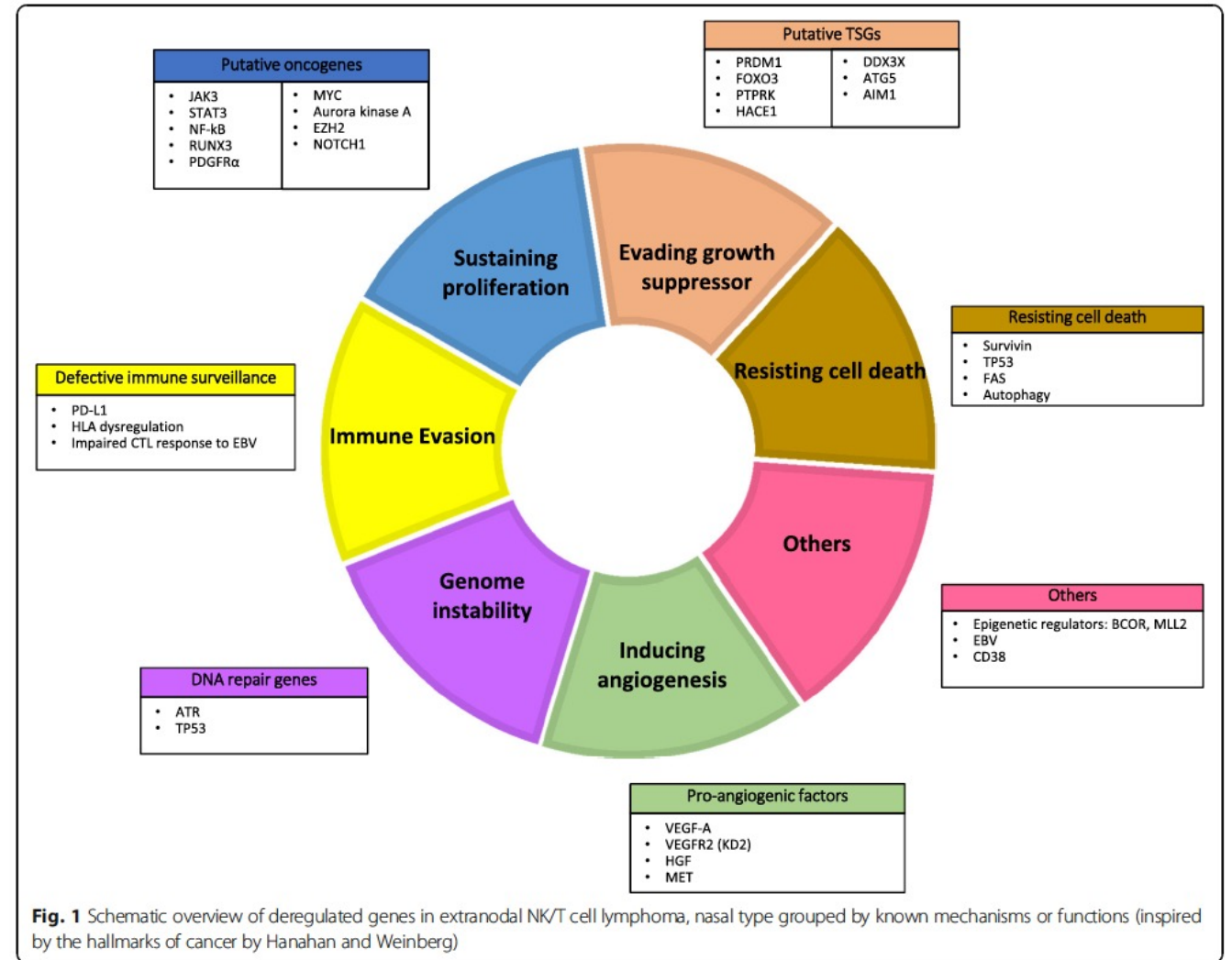
- ✓ French and Asian samples
- ✓ WES data then targeted sequencing
- ✓ Collaboration: Pr K Kataoka, Pr S Ogawa(Tokyo)

MOLECULAR ONCOGENESIS

Most frequently mutated genes in ENKTCL

STAT3	Related to JAK/STAT activation	14.2% [4-26]
DDX3X	RNA helicase	13.8% [4-21]
TP53	Tumor suppressor gene	10.4% [5-16]
BCOR	Epigenetic modifier	7.7% [2.8-32]

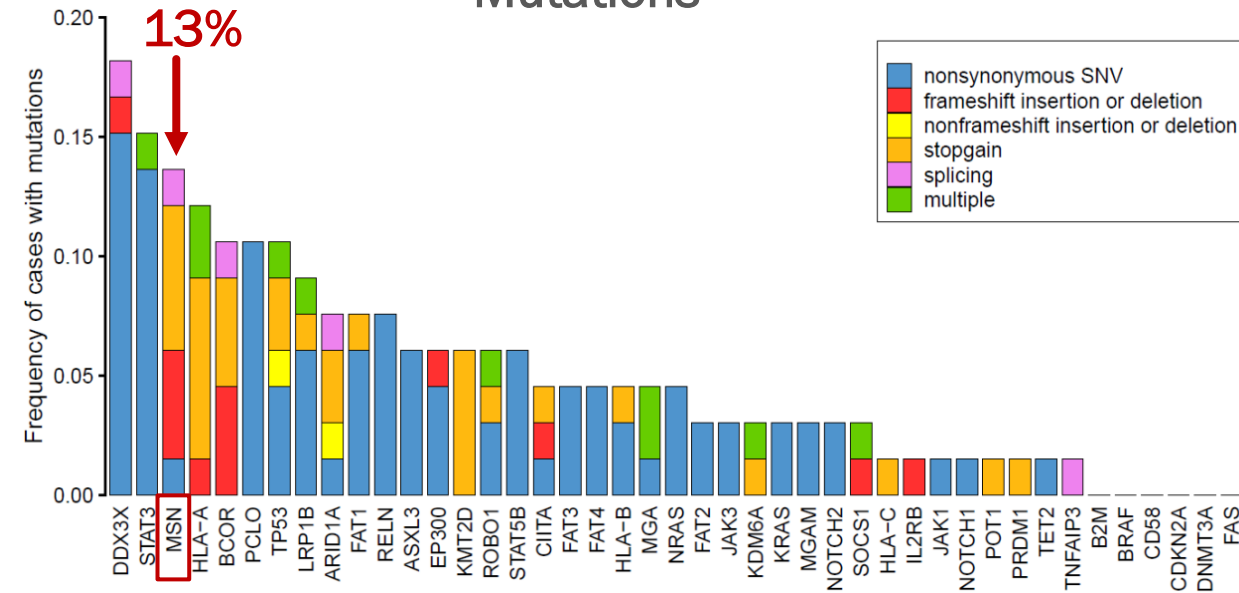
Küçük, Nat Commun, 2015
Jiang, Nat Genet, 2015
Dobashi, Gene Chromosome Cancer, 2016
Lee, Oncotarget, 2015
Song, Blood, 2018
Wen, Nature Med, 2018
Li, Nature Com, 2019
Montes-Mojarro, Mod Pathol, 2020
Xiong, Cancer Cell, 2020
Lim, Leukemia, 2020
Polprasert, Leukemia& Lymphoma, 2021



GENETIC ALTERATIONS

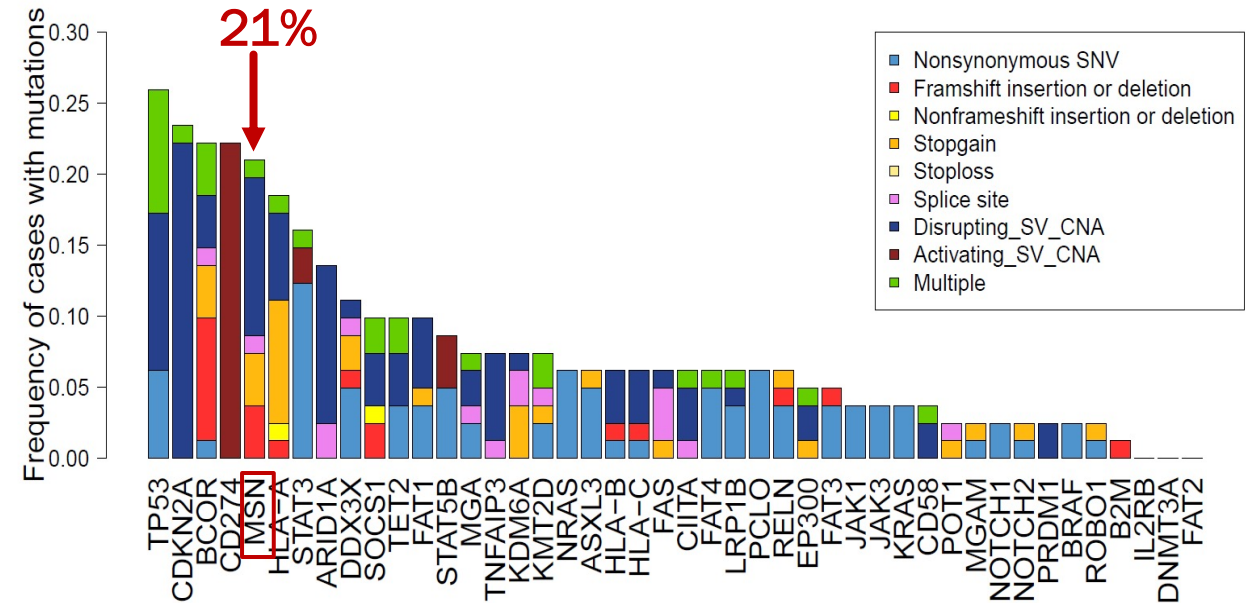
Whole exome sequencing (n=66)

Mutations



Targeted resequencing (n=81)

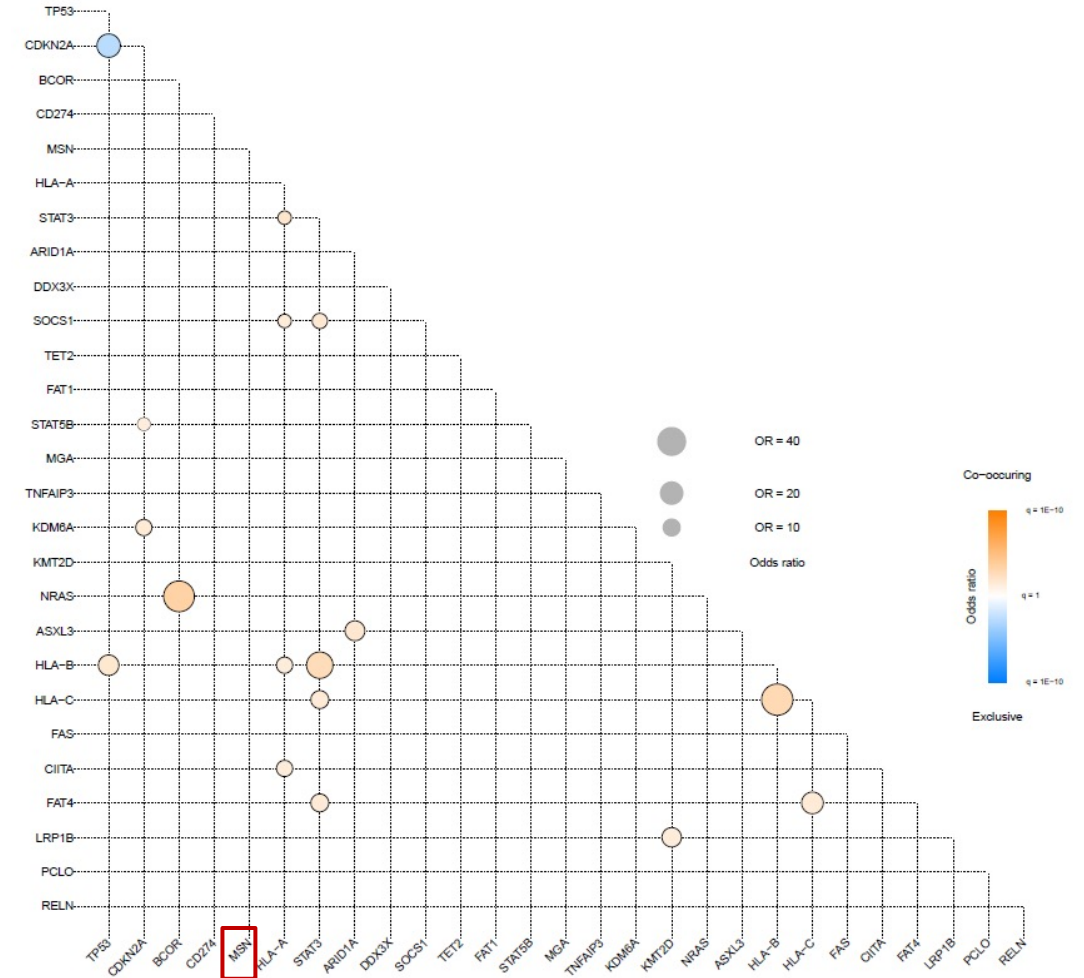
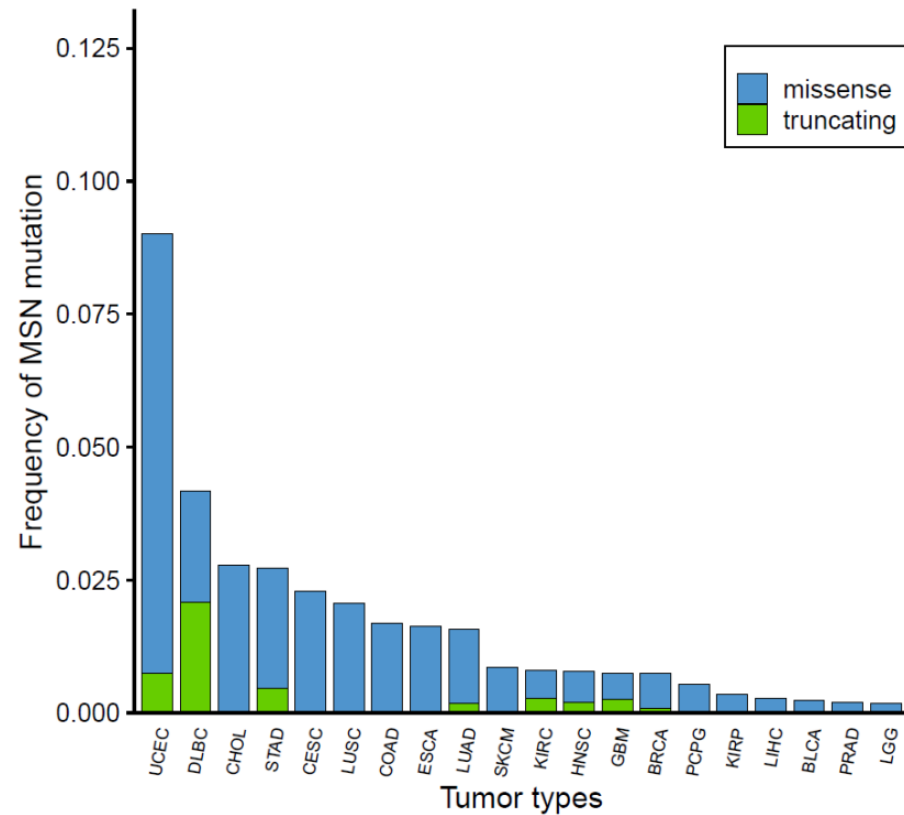
Mutations + CNV + PDL1 SV



➤ **MSN** is one of the most altered genes in ENKTCL

GENETIC ALTERATIONS

10,510 samples from 33 cancer types
(generated from open access MC3)



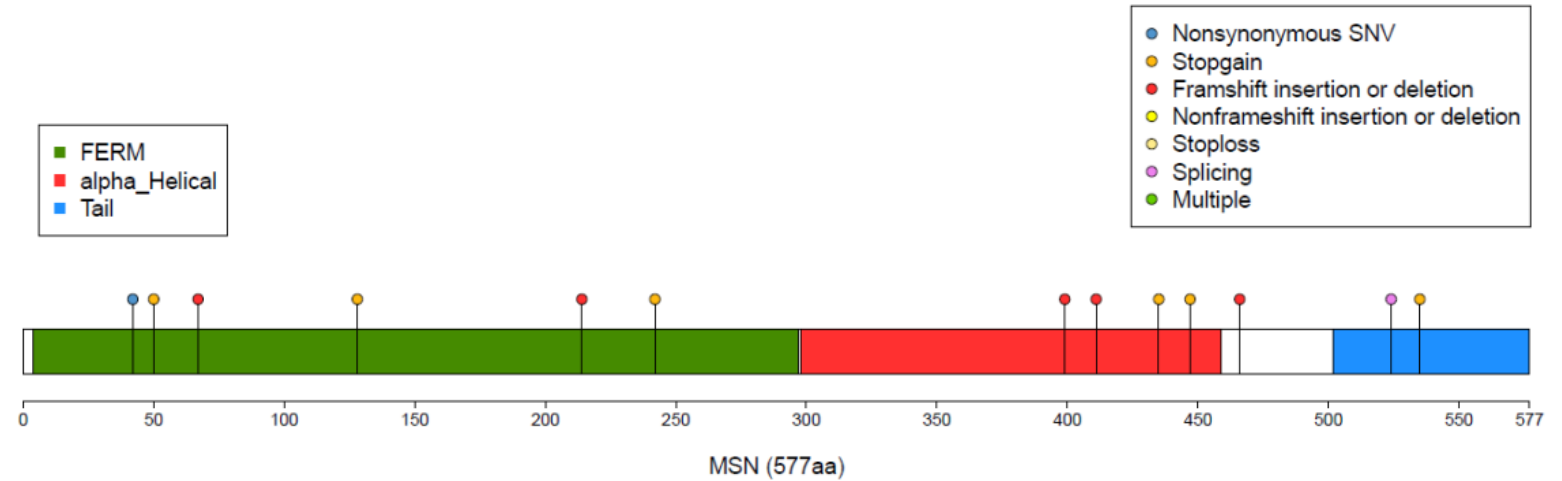
MSN mutations/ deletions are not observed in other lymphoma subsets

- **Recurrent and specific alterations in ENKTCL (TCGA)**
- **No association neither exclusion of MSN mutations with other genes**

GENETIC ALTERATIONS

Mutations

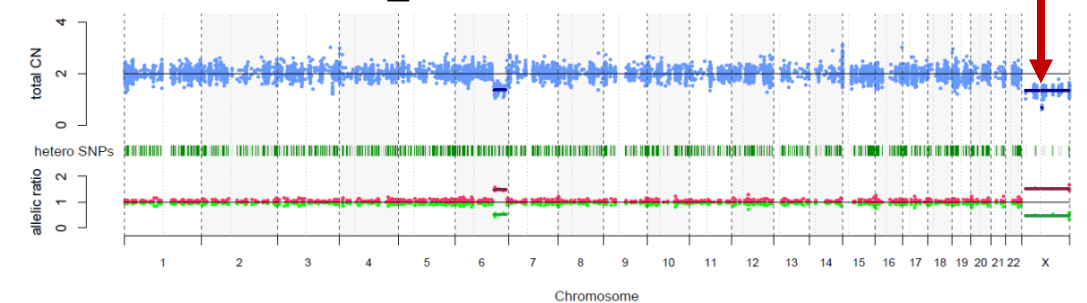
- Mutations along the gene
- **Mainly inactivating**



Deletions

- *MSN* gene is located on X chromosome
- *MSN*^{KO} by focal deletion

Homodeletion, JFCR_NKT024: Female



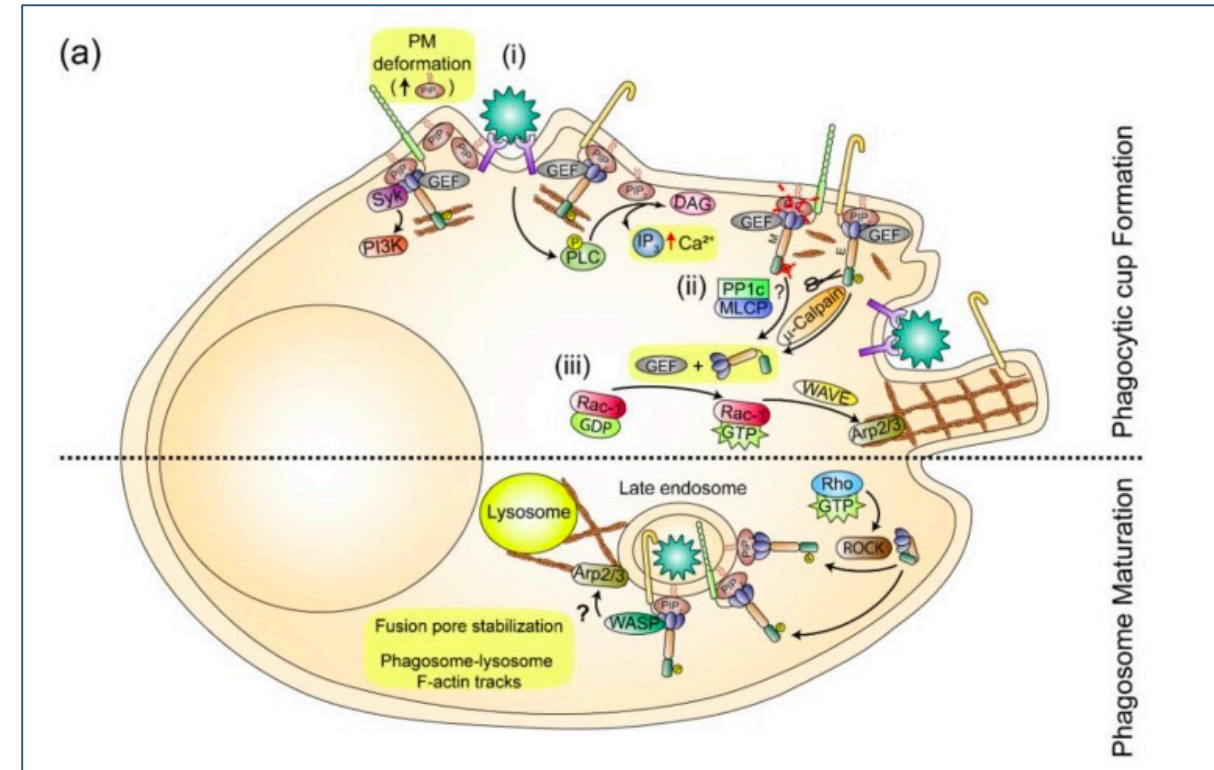
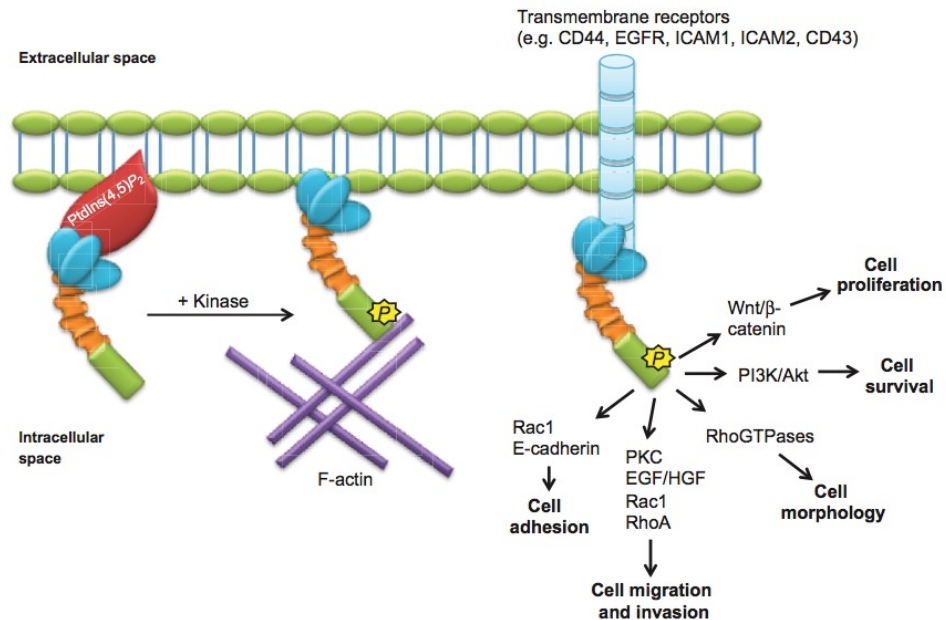
Inactivating mutations or focal deletions leads to **MSN loss of function**

➤ **MSN: tumor suppressor gene ?**

MOESIN

Physiological function

- ERM family
- Cytoskeleton protein
- Ubiquitous protein, high expression in NK lineage
- Multifunction protein: cell cortex maintenance, signal transduction, proliferation, survival, trafficking, migration and adherence when phosphorylated

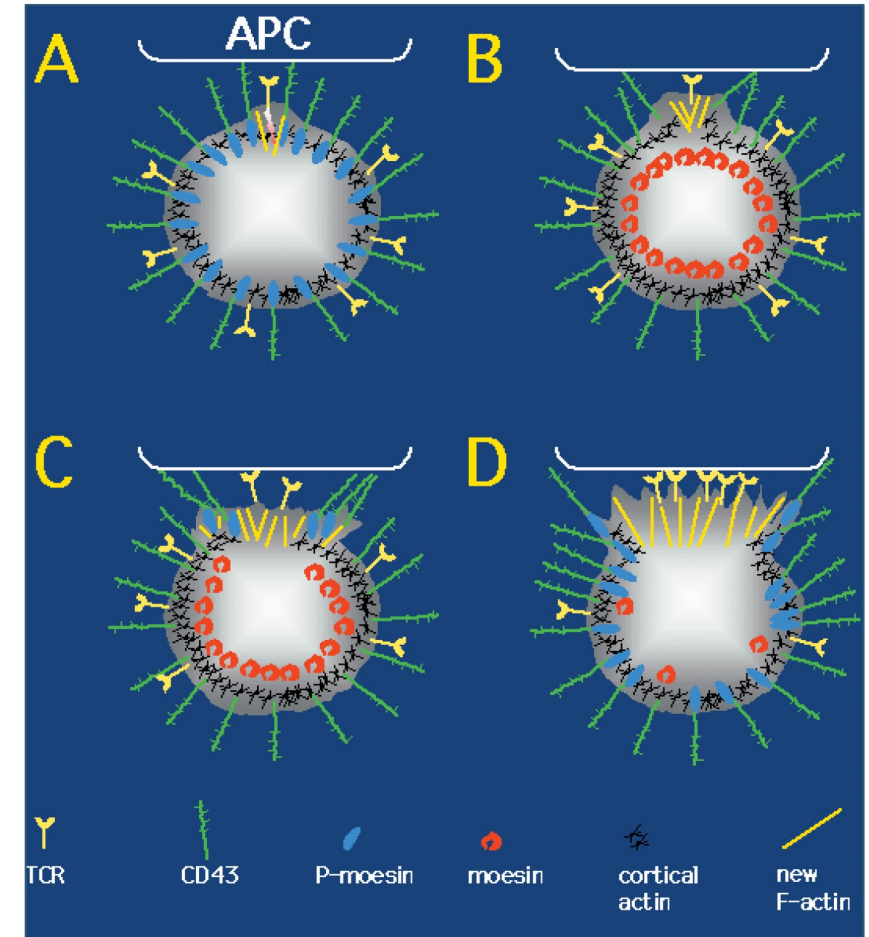
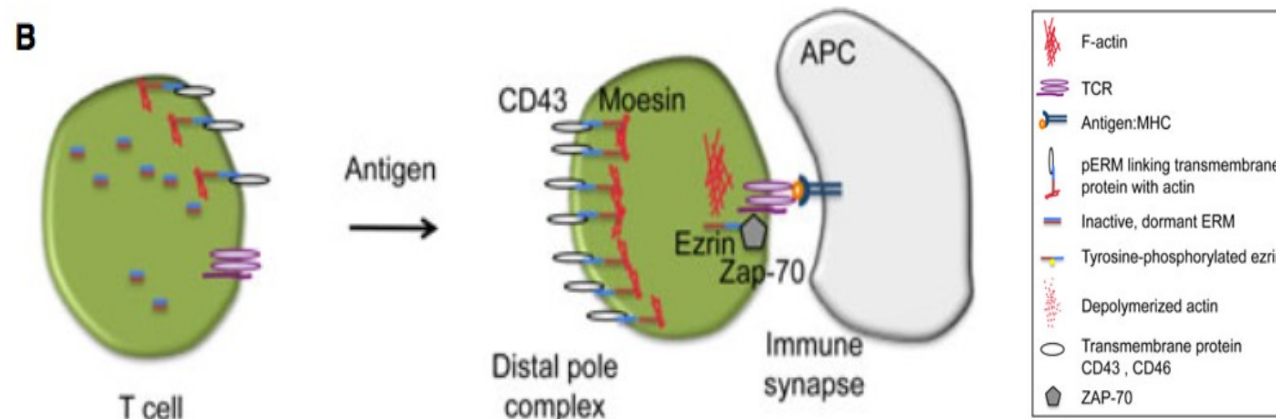


MSN play a crucial role in intracellular trafficking

MOESIN

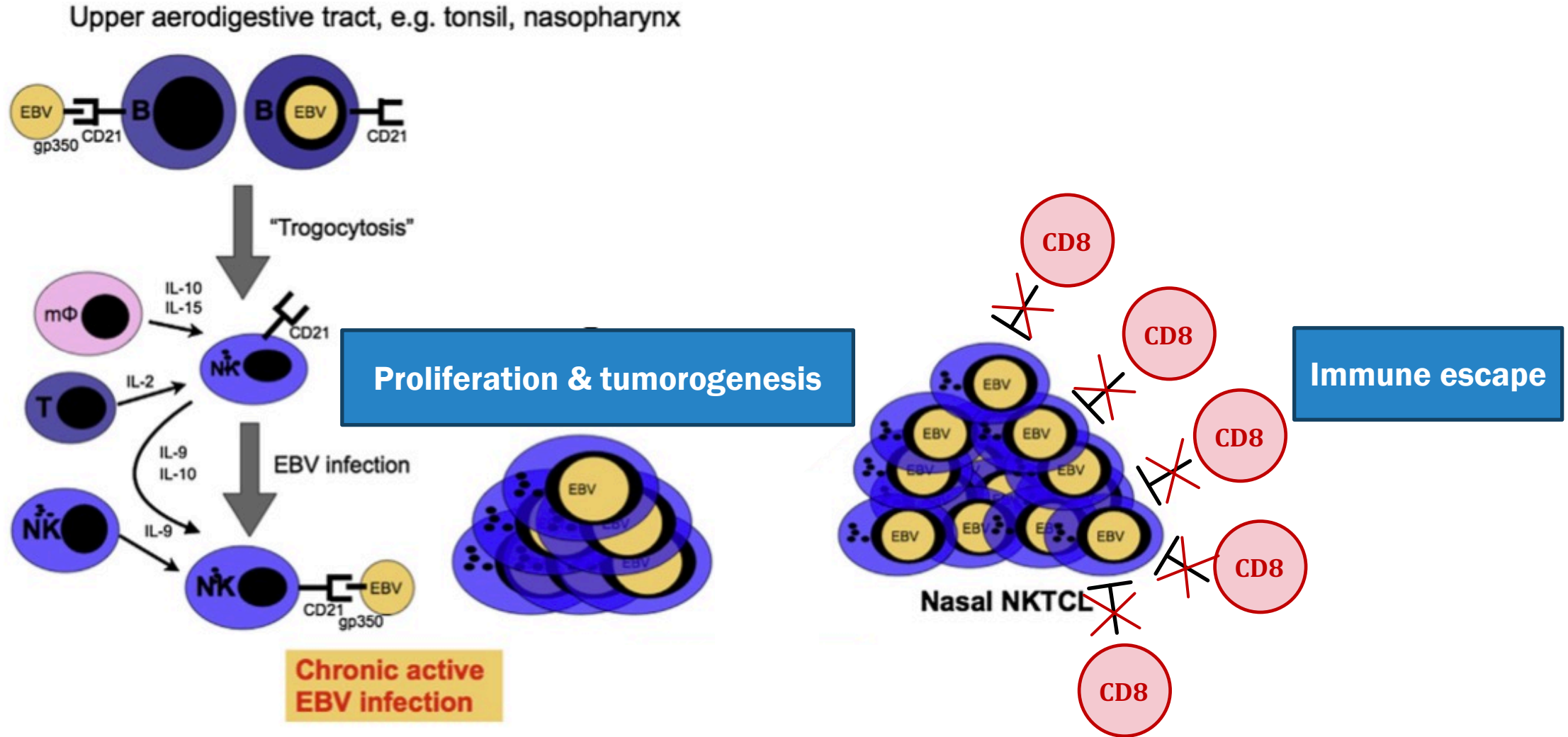
Immunological function

- Role in immune synapse formation
- Role in viral synapse HIV1/HSV1
- Germline inactivating mutations of *MSN* have been reported in 7 patients with X-linked primary immunodeficiency.

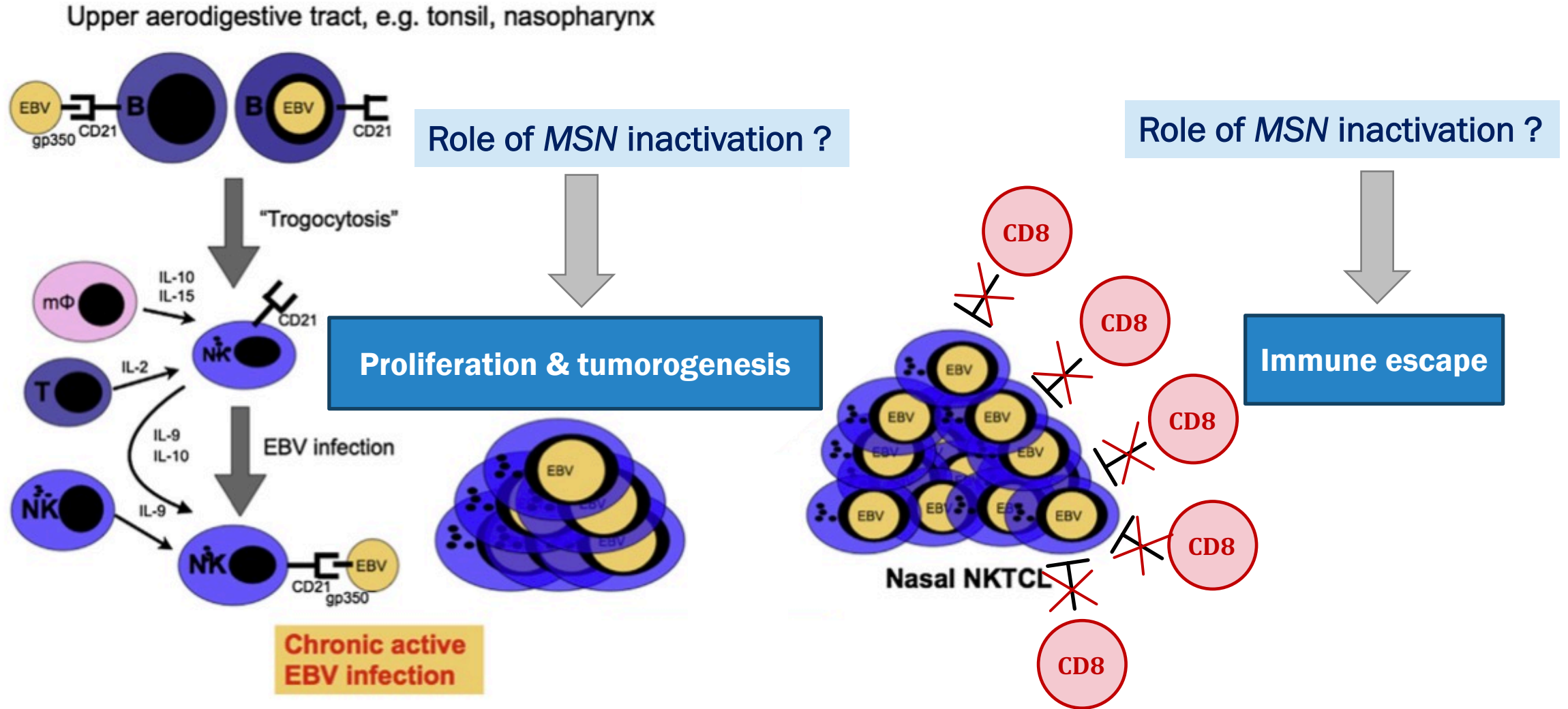


Delon, Immunological review, 2002
 Barrero-Villar, J Cell Sci, 2009
 Henning, Virology, 2011
 Parameswaran & Gupta Immunol Rev, 2013
 Clucas & Valderrama, J Cell Sci, 2015
 Lagresle-Peyrou, JACI, 2016
 Ponuwei, J Biomed Sci, 2016
 Garcia-Ortiz, Int J Mol Sci, 2020

ROLE OF MSN IN LYMPHOMAGENESIS



ROLE OF MSN IN LYMPHOMAGENESIS

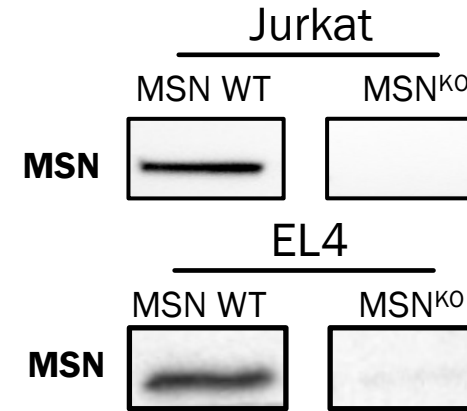
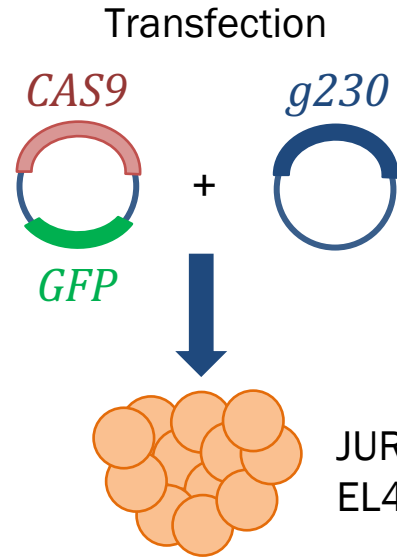


FUNCTIONAL ANALYSIS OF MSN ABNORMALITIES

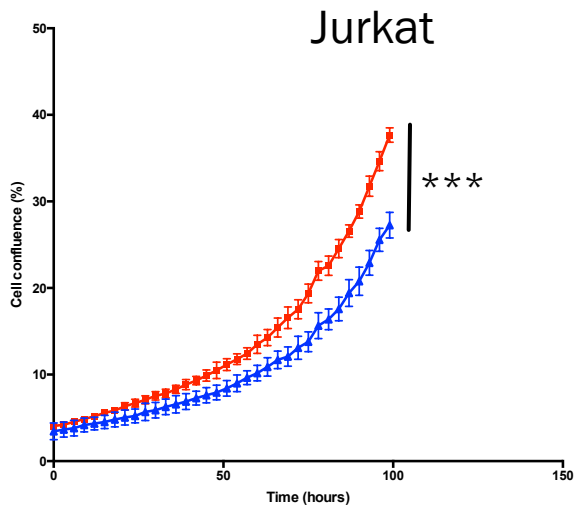
*Impact on cell transformation
and proliferation*

- ✓ Proliferation assay
- ✓ Deregulated pathways (RNAsequencing and validation assays)

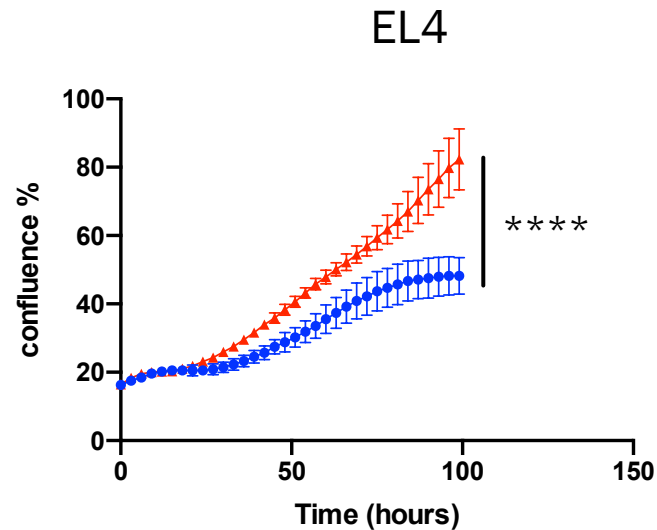
MSN & CELL PROLIFERATION



10% FBS

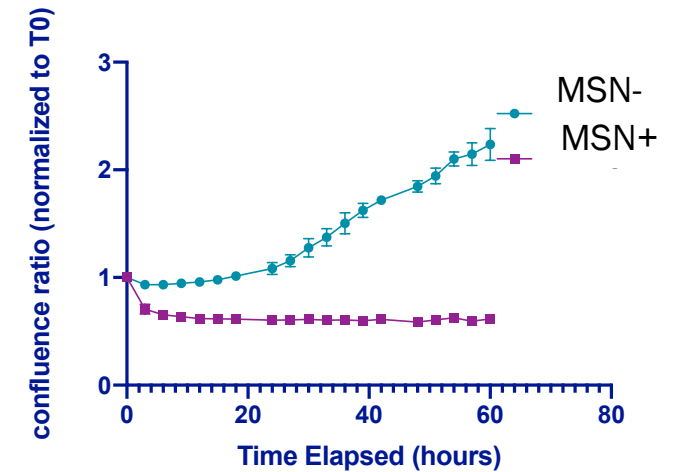


10% HS



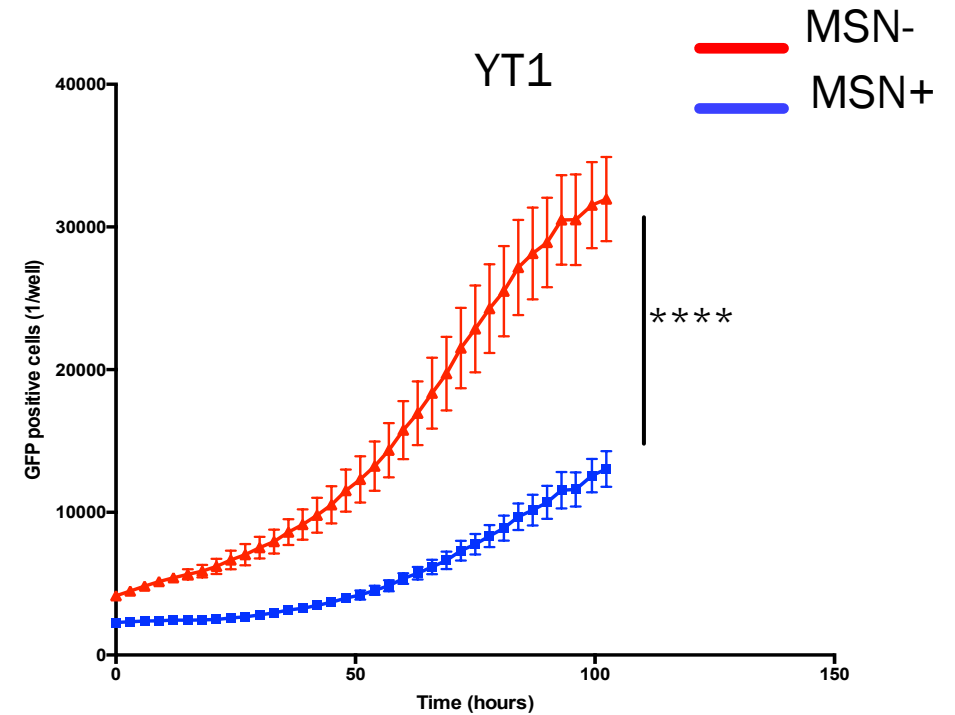
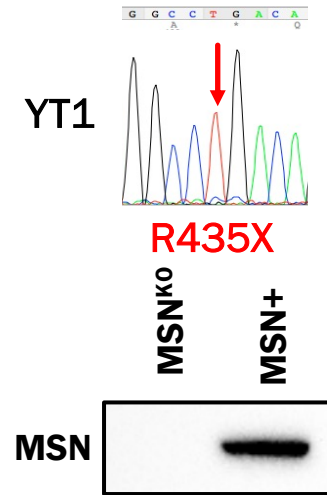
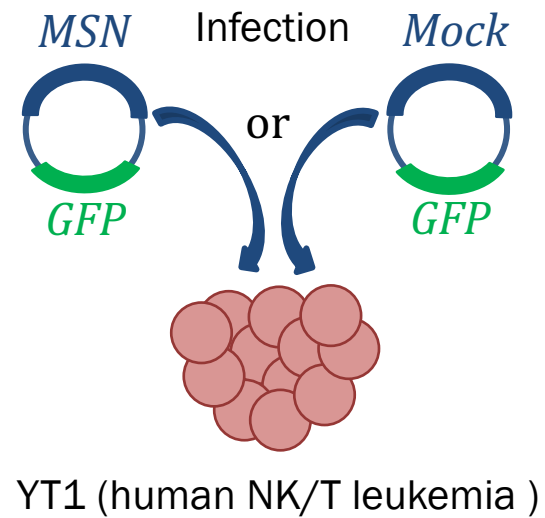
— MSN-
— MSN+

1% HS



➤ Proliferation advantage in case of *MSN* inactivation

MSN & CELL PROLIFERATION



➤ Lower rate of proliferation in case of recovered *MSN* expression

MSN & CELL SIGNALING

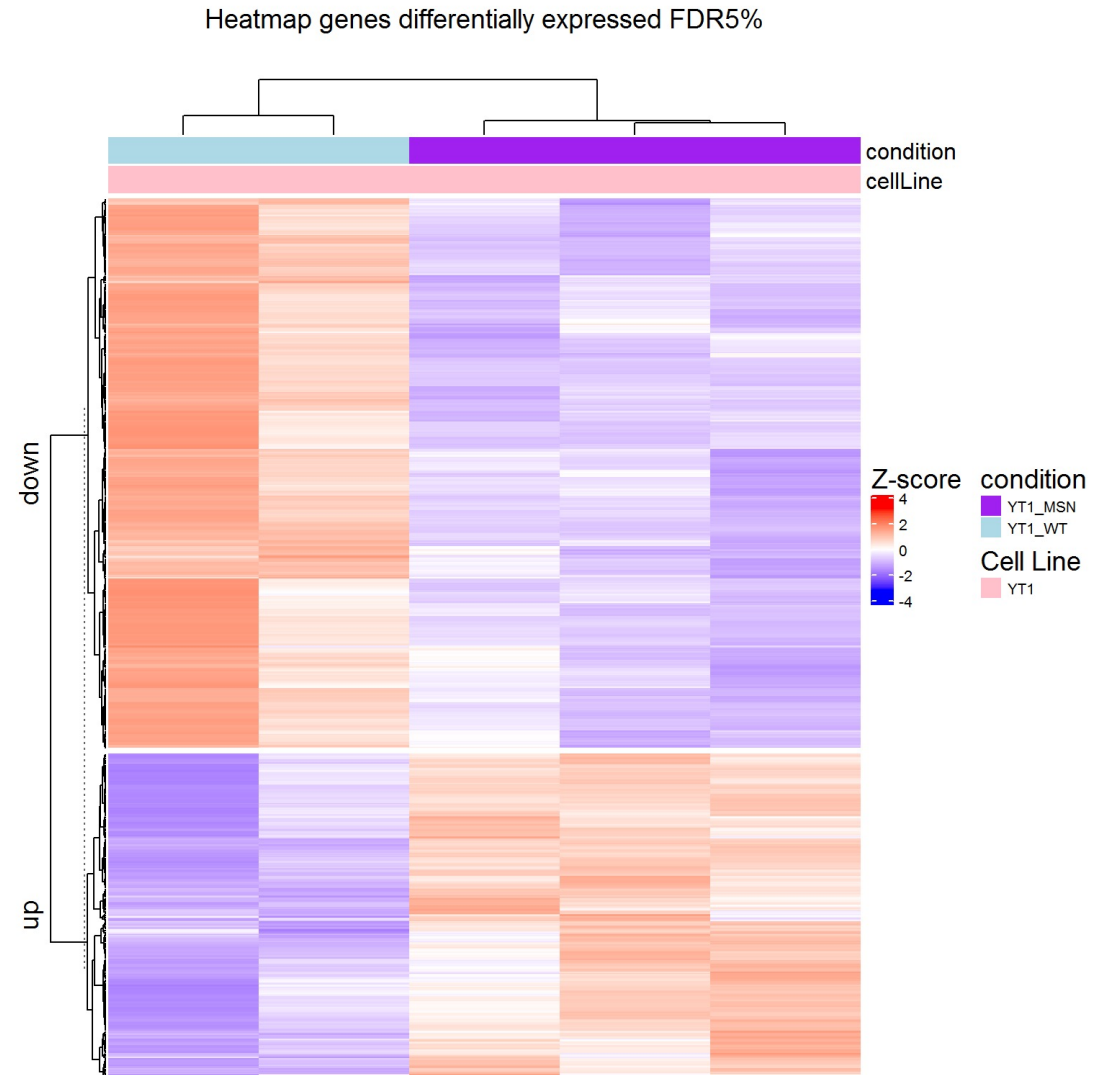
RNAseq YT1 MSN^{KO} & MSN⁺

Main upregulated genes in absence of MSN

Gene Name	P adjusted value
TNFRSF8 = CD30 (member TNF receptor superfamily)	2,18488E-08
MCM4	1,52905E-07
MYB	1,02545E-06
NFKBIA = IKBα	3,80226E-05
MCM5	5,07323E-05
MCM7	5,07323E-05
MCM2	7,51259E-05

Main pathways upregulated in absence of MSN

- **NFkB**
- **NOTCH**
- **MYC**
- **mTORC1**



NFKB PATHWAY

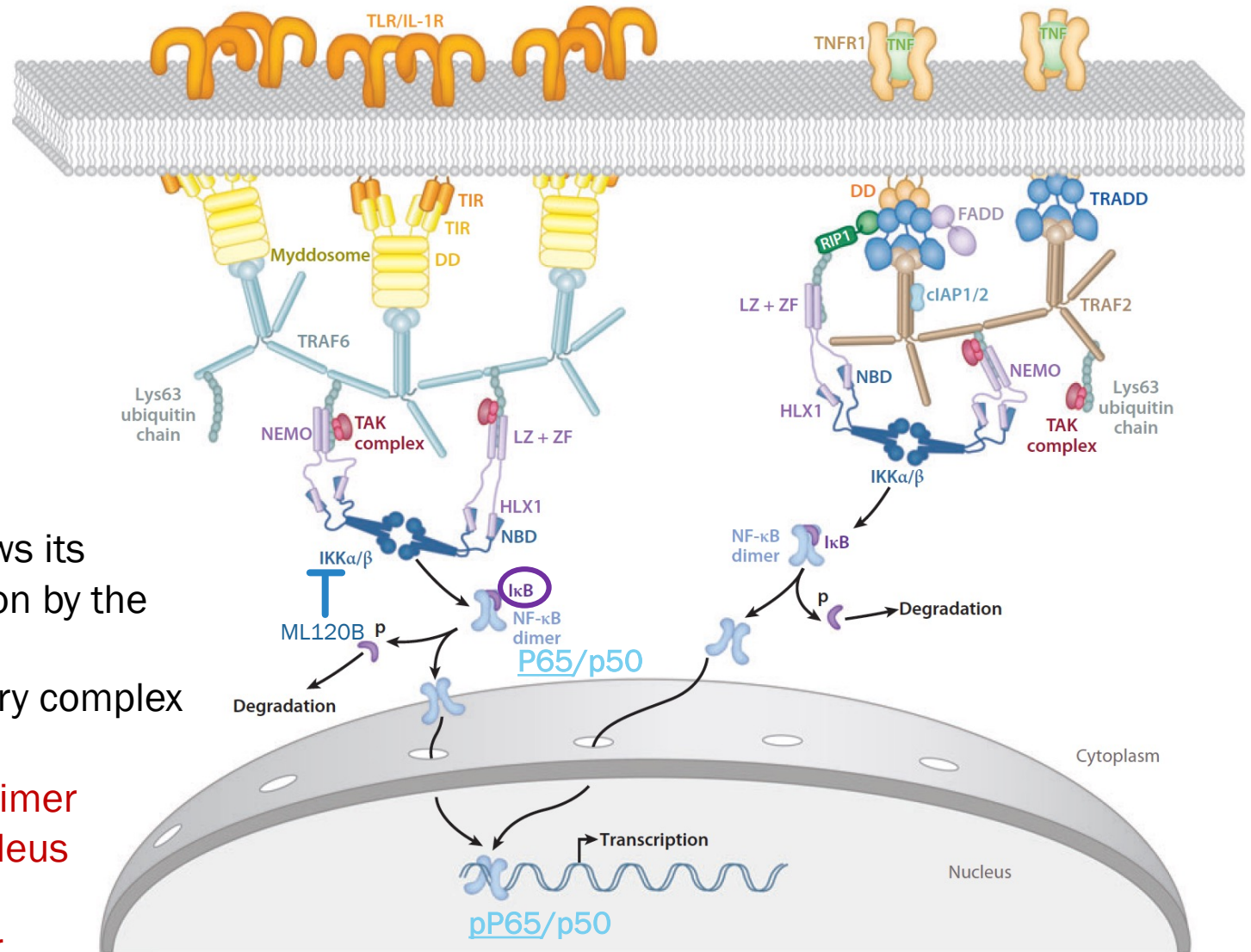
1/ Activation via TNF-R or TLR

2/ IKK complex activation leads to I κ Ba phosphorylation

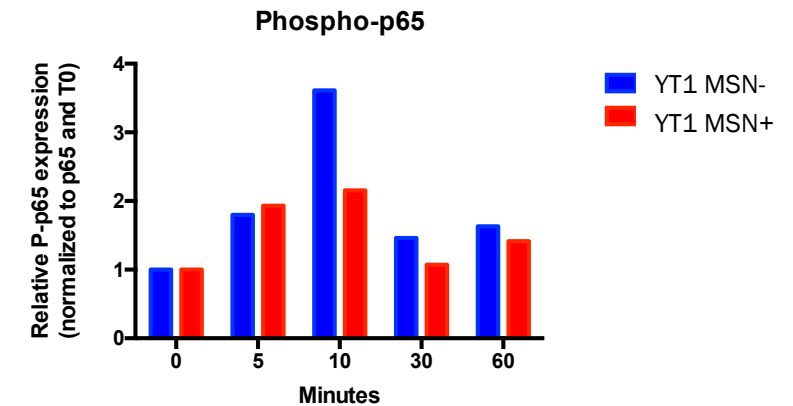
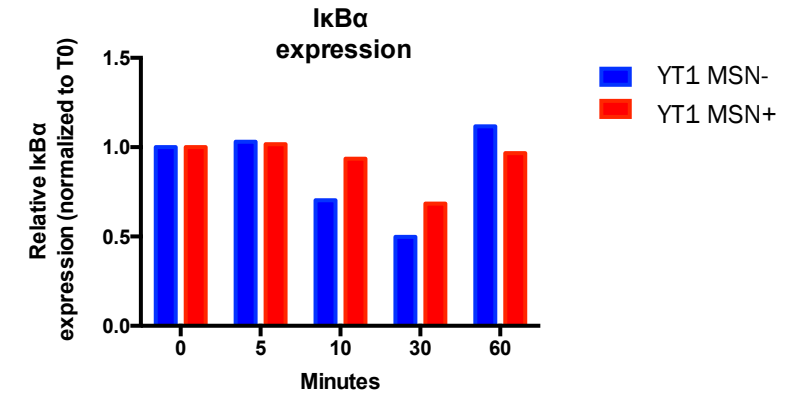
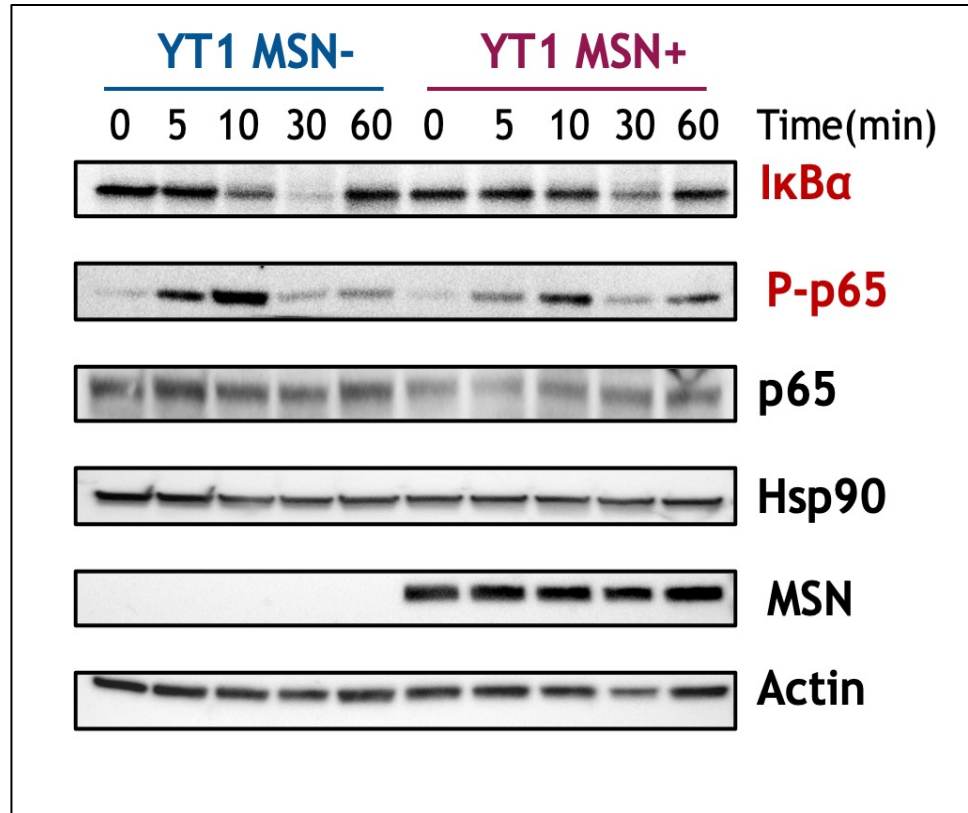
3/ I κ Ba phosphorylation allows its ubiquitination then its degradation by the proteasome.

→ NF κ B release from I κ Ba inhibitory complex

4/ NF κ B homo or heterodimer translocation into the nucleus
→ DNA binding
→ Transcription factor

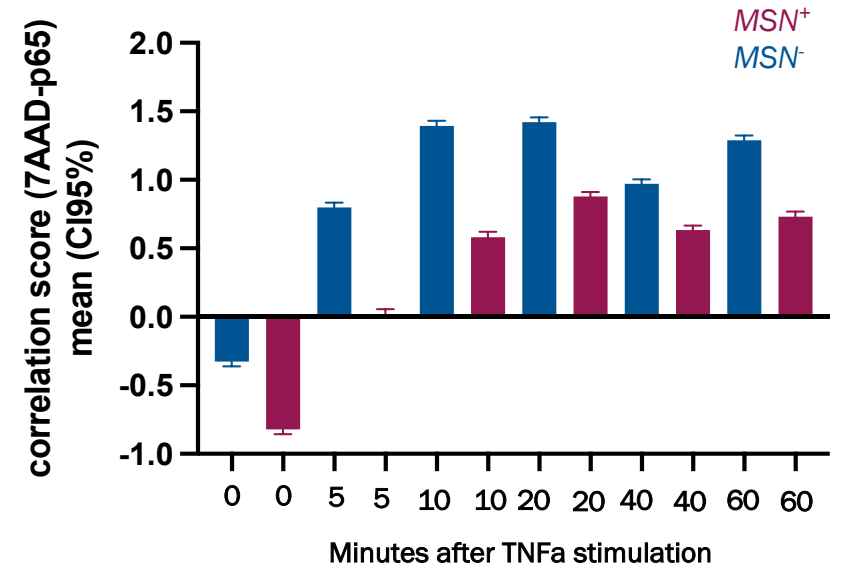
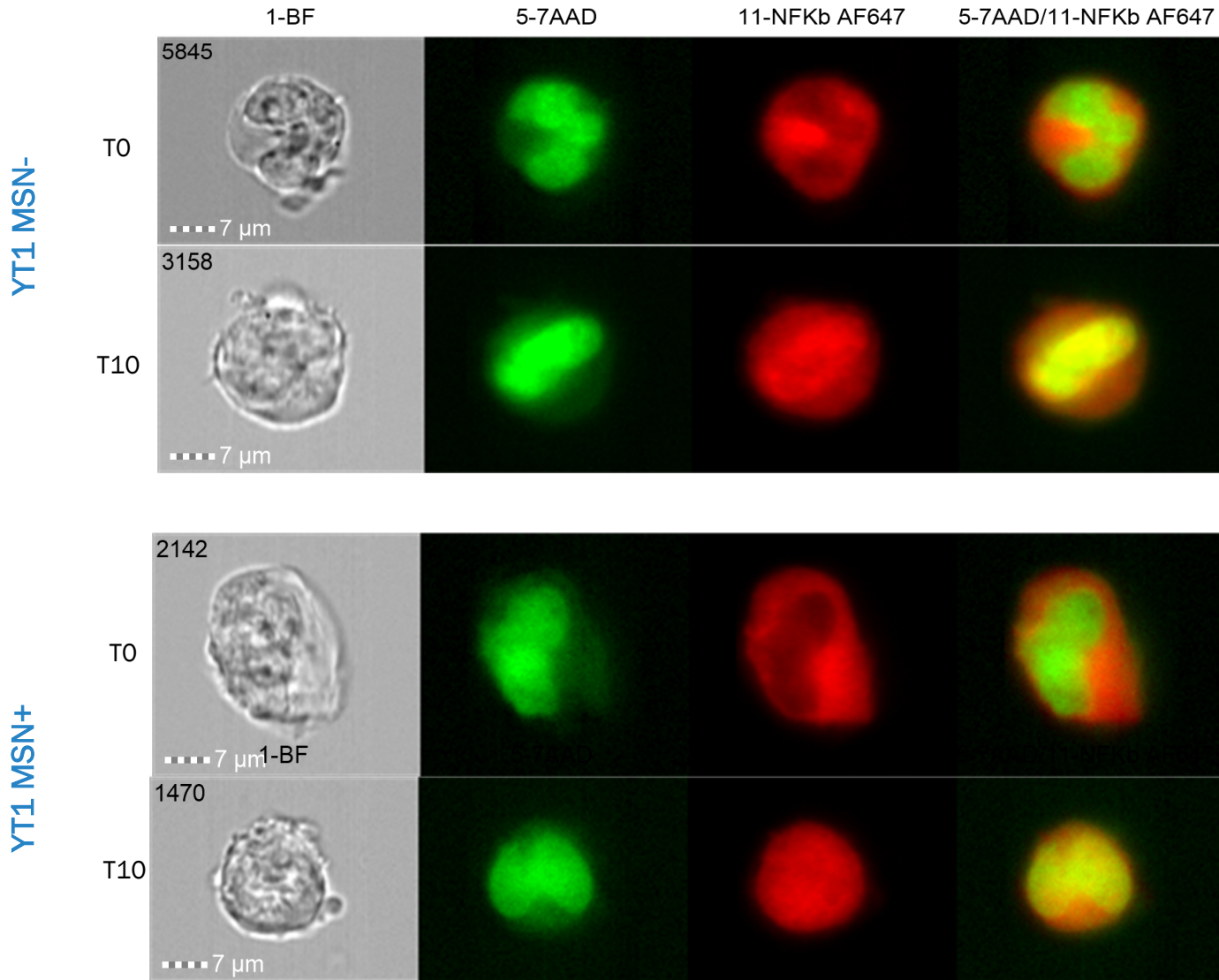


MSN & NFKB PATHWAY



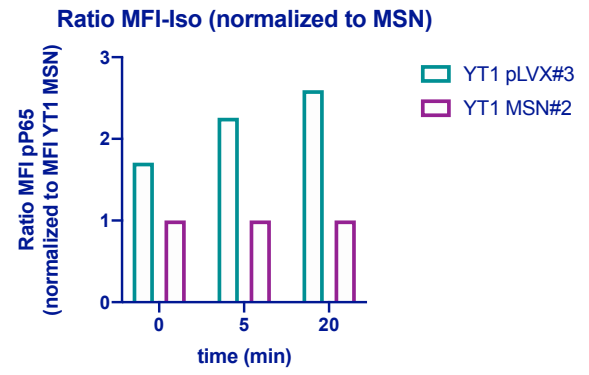
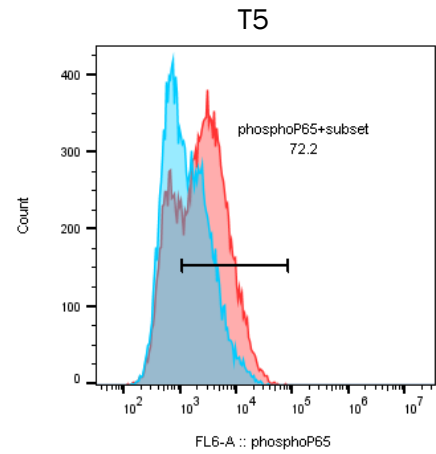
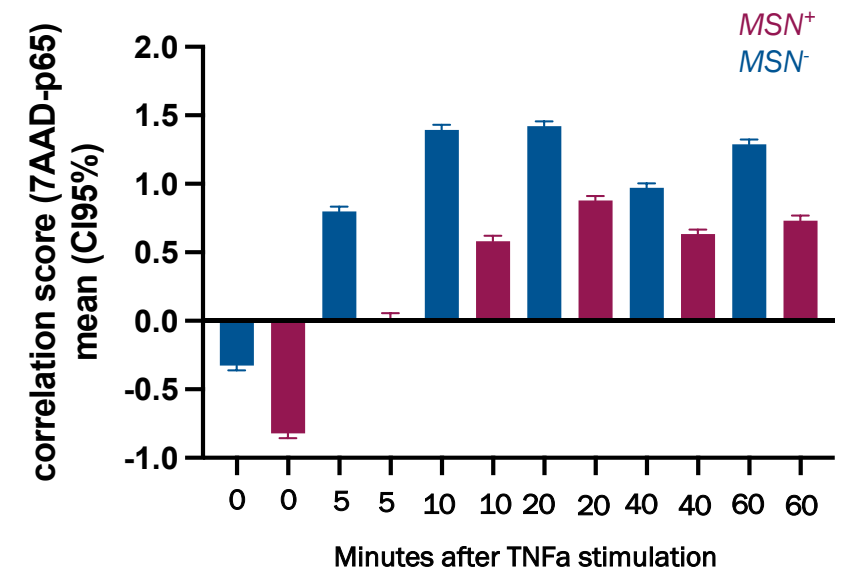
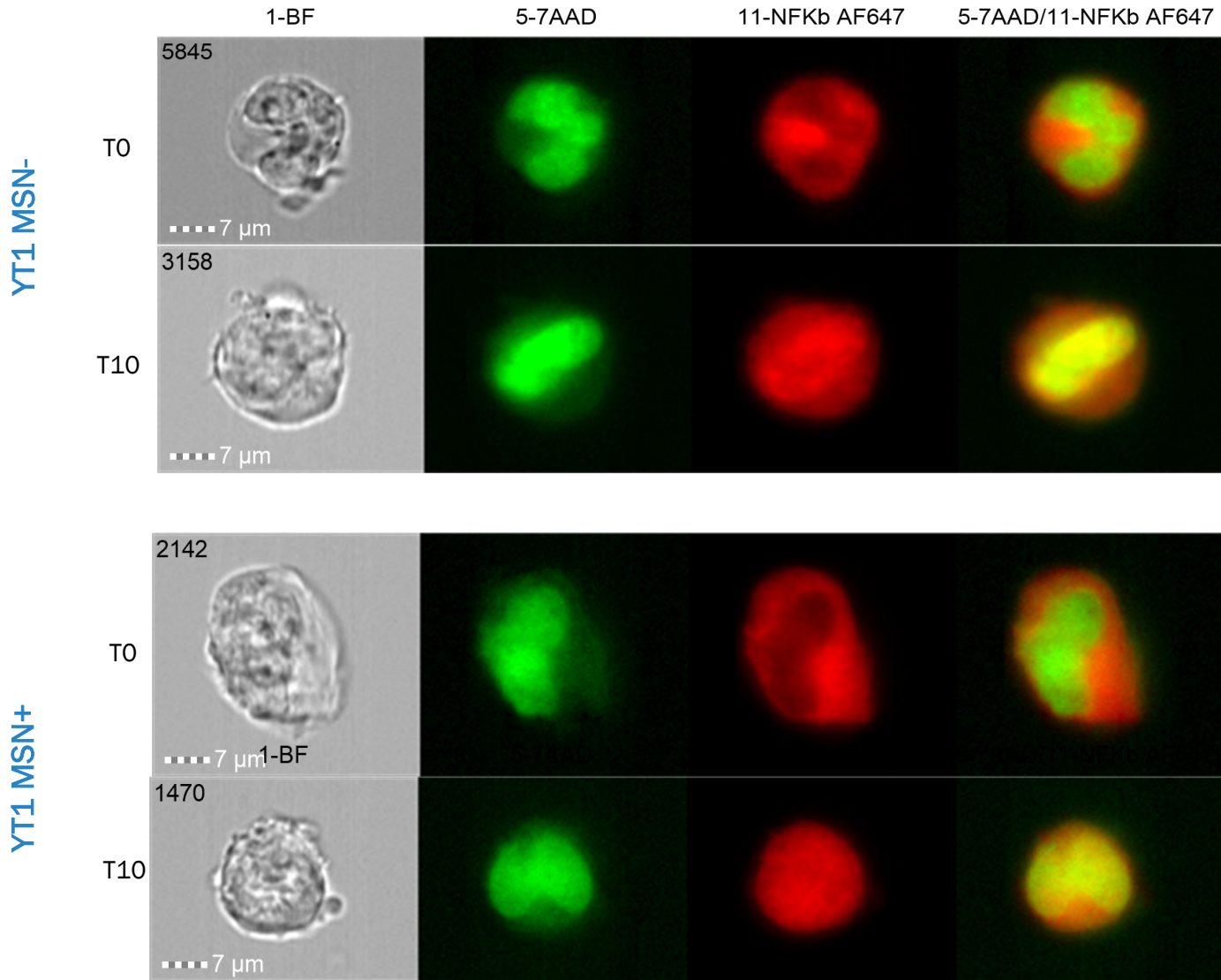
- **MSN inactivation in YT1 cells is associated with an increase of IκBα degradation and higher level of phosphoP65 expression upon TNFα stimulation suggesting a canonical activation of NFκB**

MSN & NFKB PATHWAY



Representative of 3 experiments

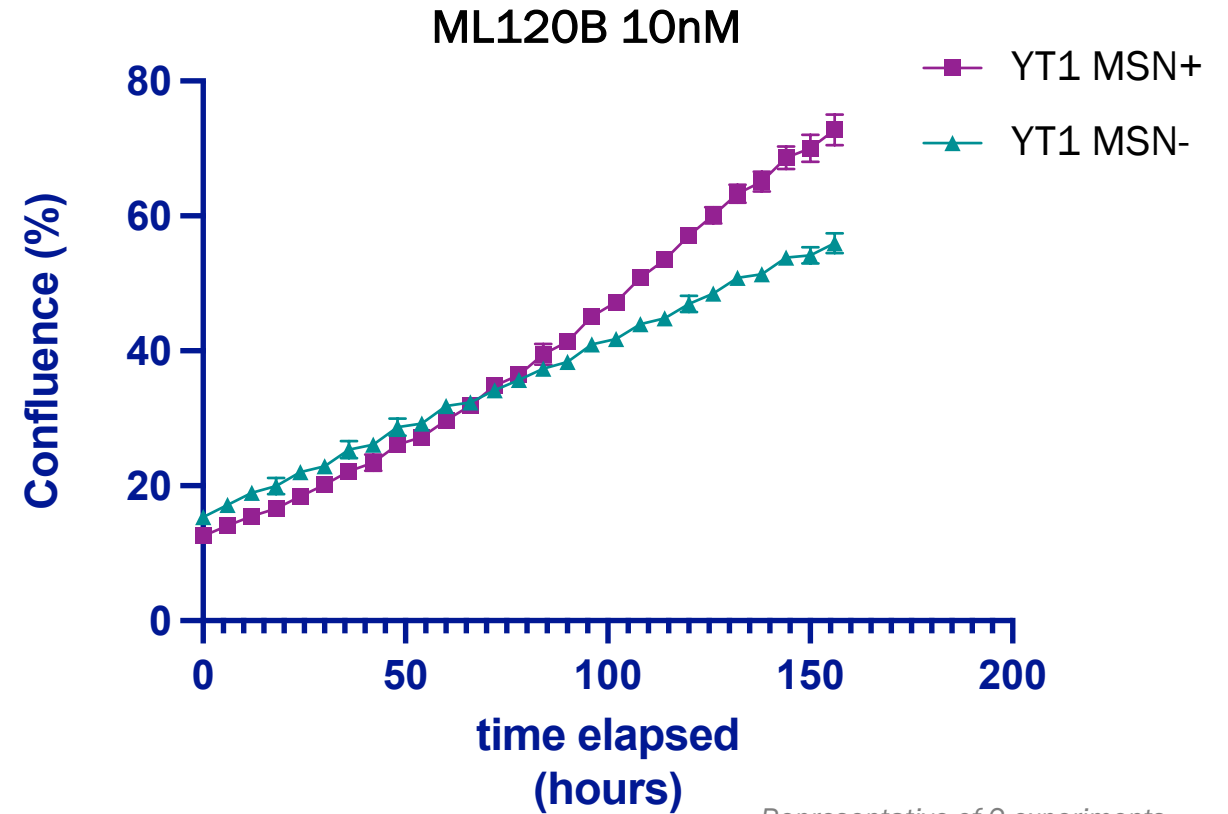
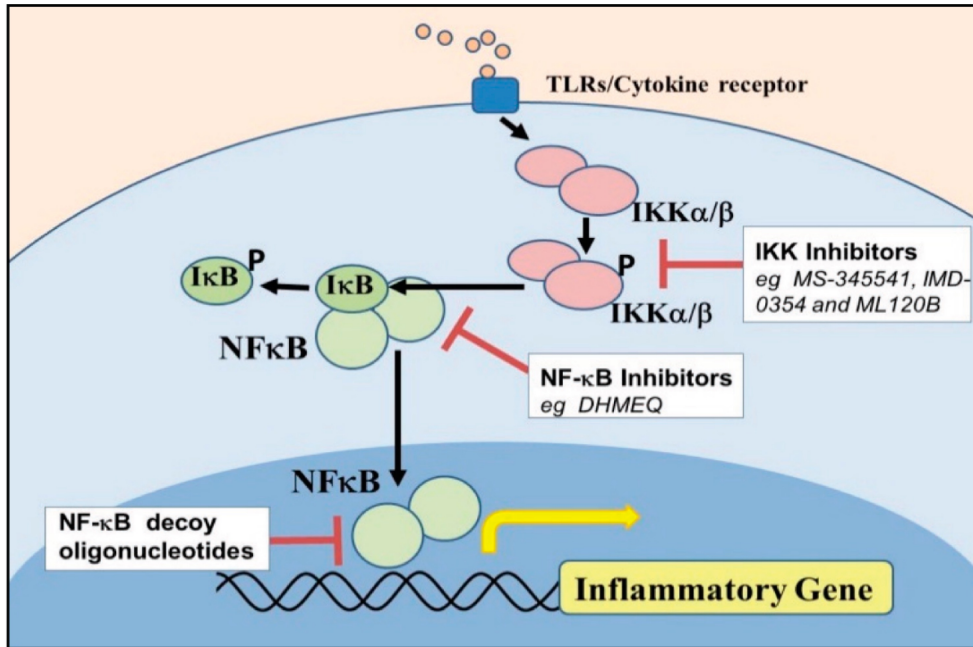
MSN & NFKB PATHWAY



➤ Significant increase of p65 translocation into the nucleus after TNFa stimulation in MSN^{KO} cells

MSN & NFKB PATHWAY

- Assessment of NFκB inhibition

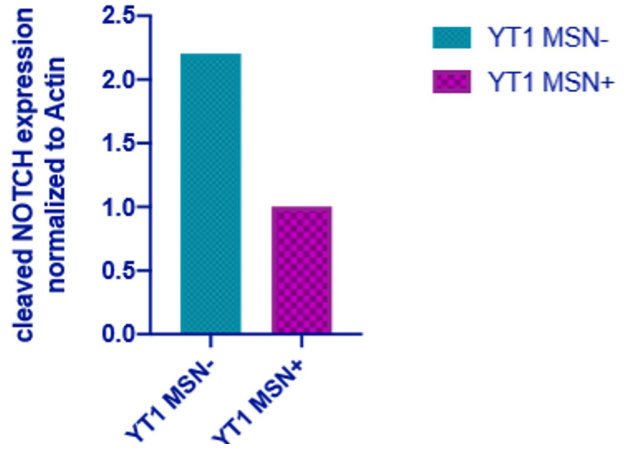
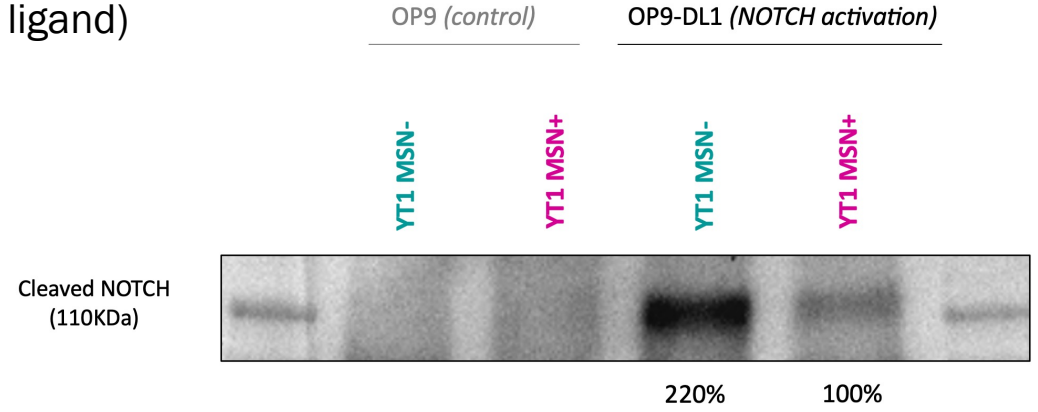


Representative of 2 experiments

➤ MSN^{KO} cells are more sensitive to ML120B (IKK complex inhibitor) as compared to MSN^{+} cells

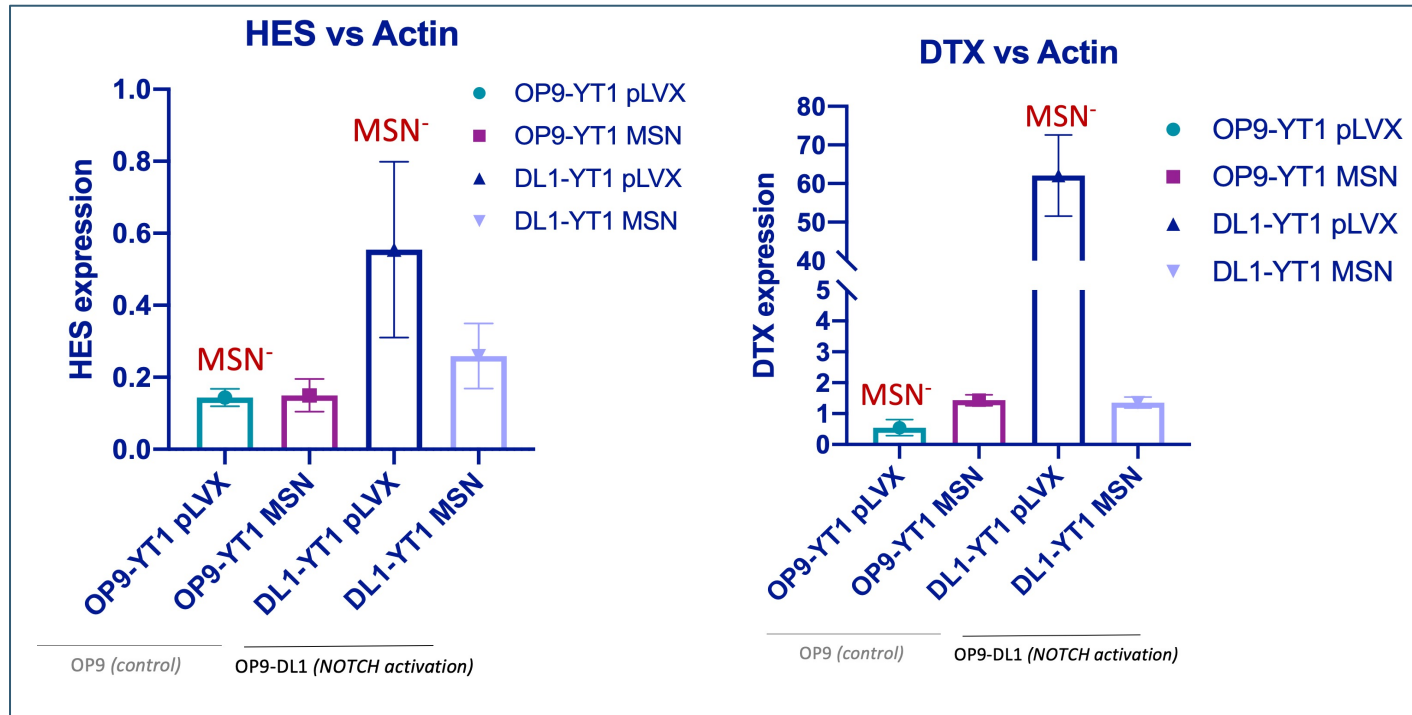
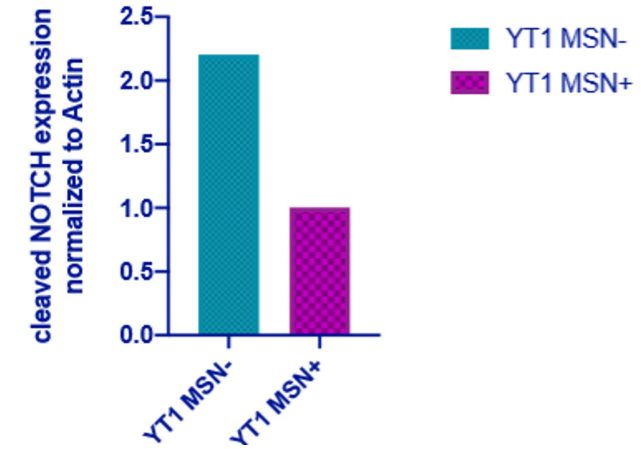
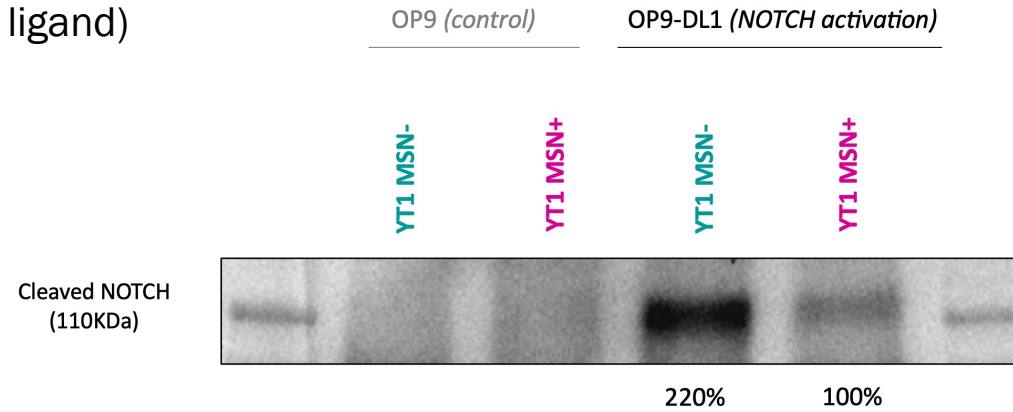
MSN & NOTCH PATHWAY

- Low basal activation of NOTCH pathway in YT1 cell line
- Need co-culture on OP9-DL1 (NOTCH ligand)



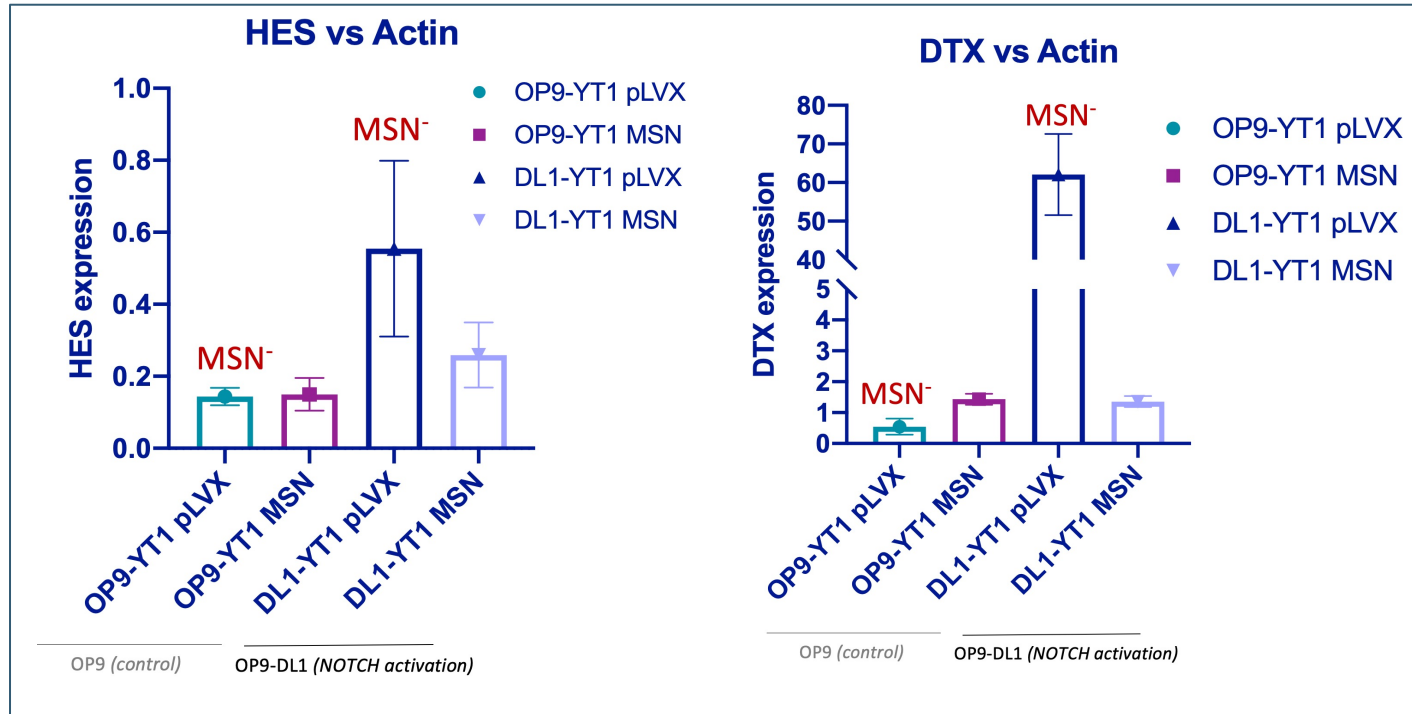
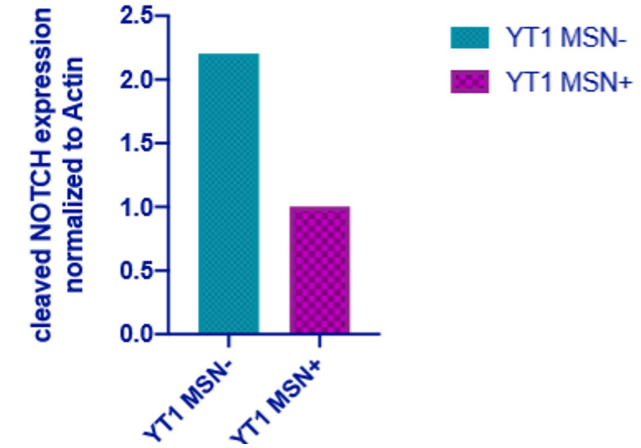
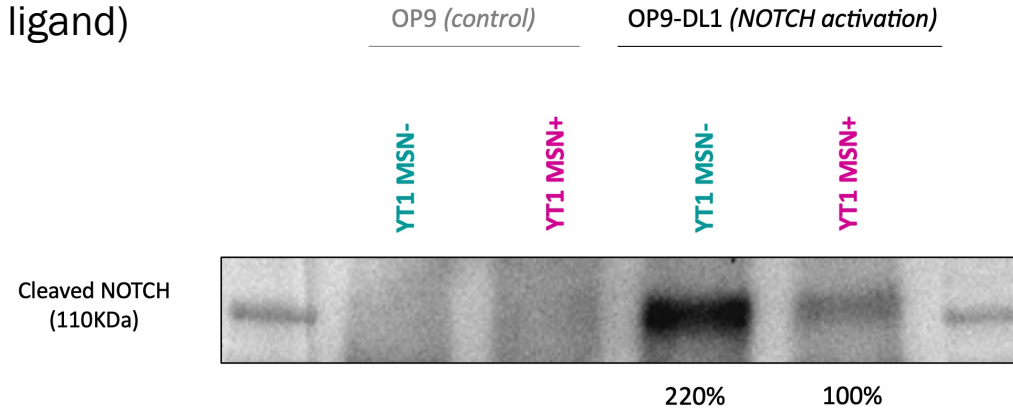
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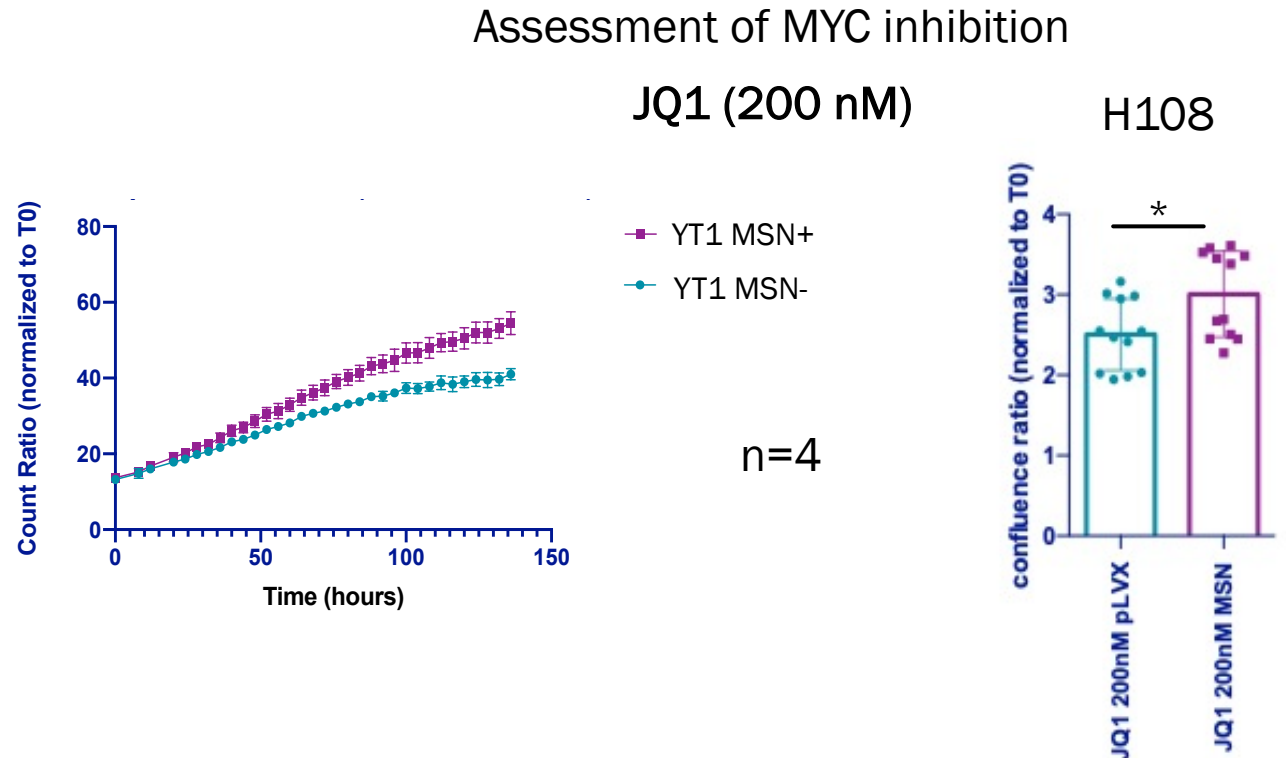
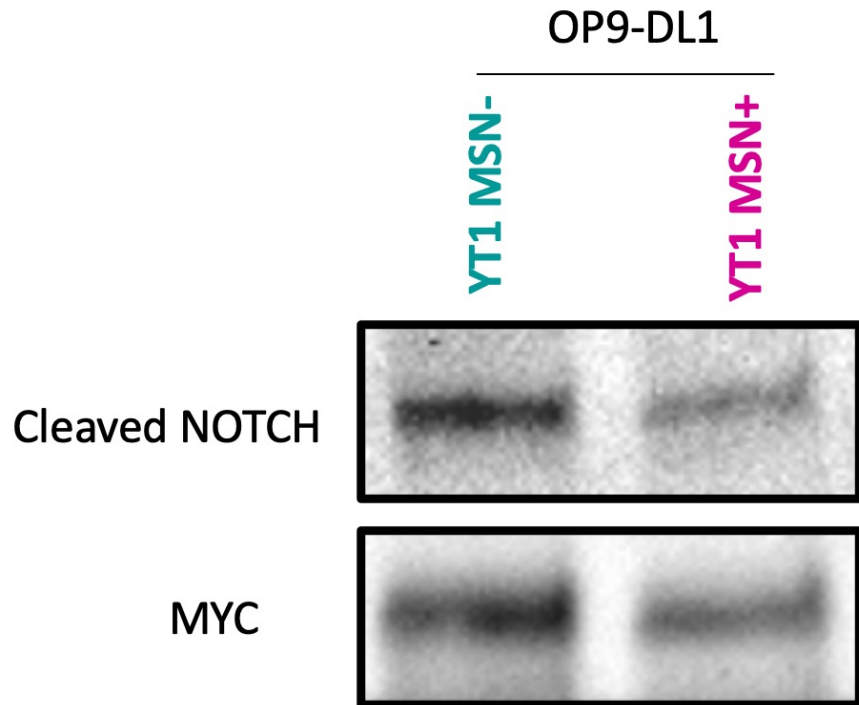
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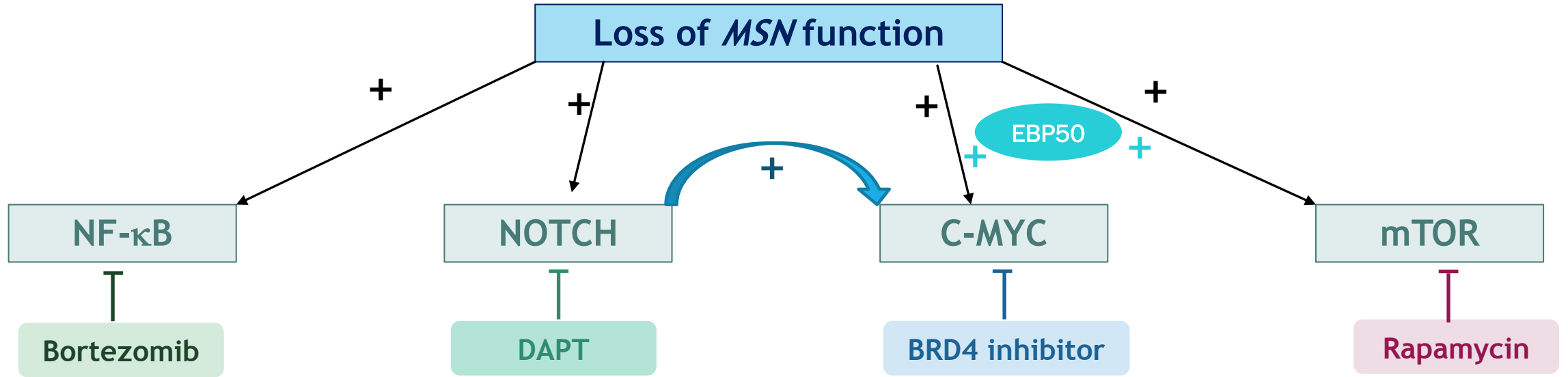
➤ NOTCH pathway is upregulated in case of MSN inactivation as compared to MSN⁺ cells after co culture with OP9-DL1

MSN & MYC PATHWAY



- *MSN* inactivation leads to NOTCH and MYC activation in YT1 cell line
- YT1 *MSN*^{KO} are more sensitive to MYC inhibition using JQ1 as compared to YT1 *MSN*⁺
- MYC staining (IHC) on patients tumor samples is *ongoing*

PROSPECTS FOR TARGETED THERAPIES



IMMUNE ESCAPE

*Investigate the role of MSN
inactivation in ENKTCL
immune evasion*

- ✓ Demonstrate that *MSN* loss of function could contribute to immune escape.

- ✓ Mechanisms involved ?
 - PDL1 and PDL2 expression
 - Immune synapse stability
 - Other ?

ENKTCL & IMMUNE EVASION : WHAT IS KNOWN ?

- ENKTCL = EBV+ tumor, affects patients **without immune deficiency**
- Mechanisms possibly involved in immune evasion :
 - **EBV Epitope alterations** (LMP1 & LMP2A) => less immunogenicity of EBV strains
(Demachi-Okamura, Eur J Immunol, 2006; Nagamine, Virus Genes, 2007; Nagamine, Intervirology, 2007; Wang, J Gen Virol, 2010, Palser, J Virol 2015)
 - **PD-L1 upregulation** induced by EBV (LMP1) and JAK/STAT activation (STAT3)
(Bi, J Hematol Oncol, 2016; Song, Blood, 2018)
 - Mutations in **immunosurveillance genes** (30% of the patients)
(Polprasert, Leukemia lymphoma, 2020)

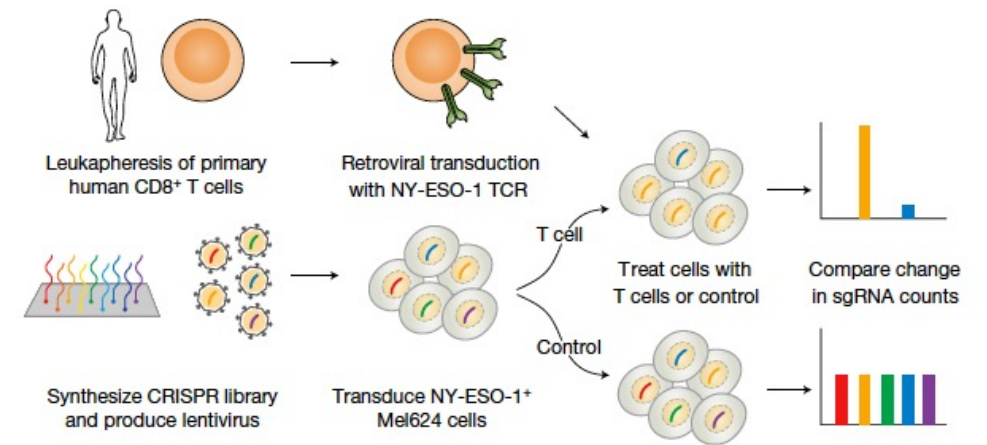
MOESIN AS A CRUCIAL GENE REQUIRED FOR ANTI-TUMOUR IMMUNE RESPONSE ?

- MSN has been described as an essential gene in target cells for T mediated cytotoxicity

(Patel, Nature, 2017)

Identification of essential genes for cancer immunotherapy

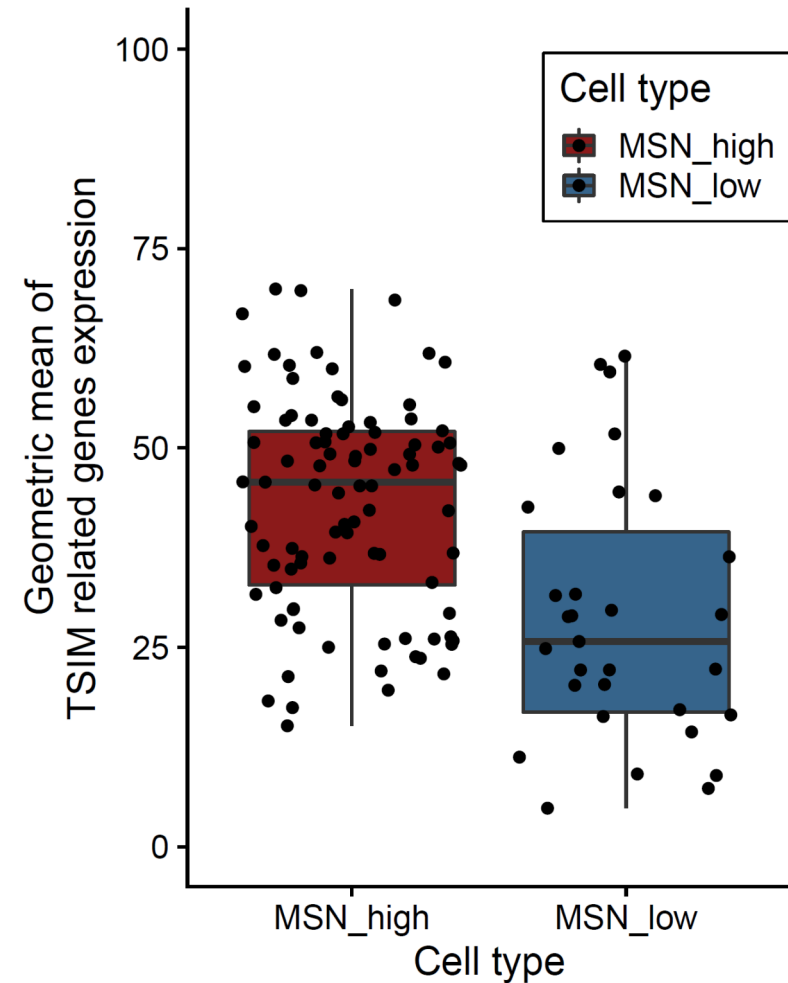
Shashank J. Patel^{1,2*}, Neville E. Sanjana^{3,4*}, Rigel J. Kishton¹, Arash Eidizadeh¹, Suman K. Vodnala¹, Maggie Cam¹, Jared J. Gartner¹, Li Jia¹, Seth M. Steinberg¹, Tori N. Yamamoto^{1,5}, Anand S. Merchant¹, Gautam U. Mehta¹, Anna Chichura¹, Ophir Shalem⁶, Eric Tran¹, Robert Eil¹, Madhusudhanan Sukumar¹, Eva Perez Guijarro¹, Chi-Ping Day¹, Paul Robbins¹, Steve Feldman¹, Glenn Merlino¹, Feng Zhang^{7,8} & Nicholas P. Restifo^{1,9}



- **MSN** loss of function may be involved in resistance to immunotherapy ?

MOESIN AS A CRUCIAL GENE REQUIRED FOR ANTI-TUMOUR IMMUNE RESPONSE ?

- MSN has been described as an essential gene in target cells for T mediated cytotoxicity
(Patel, Nature, 2017)
- TSIM subtype (associated with antigen presentation, PD1 expression and JAK STAT activation): less represented in MSN^{low} group



Brunner-Munzel test p<0.01

- **MSN loss of function may be involved in immune evasion**

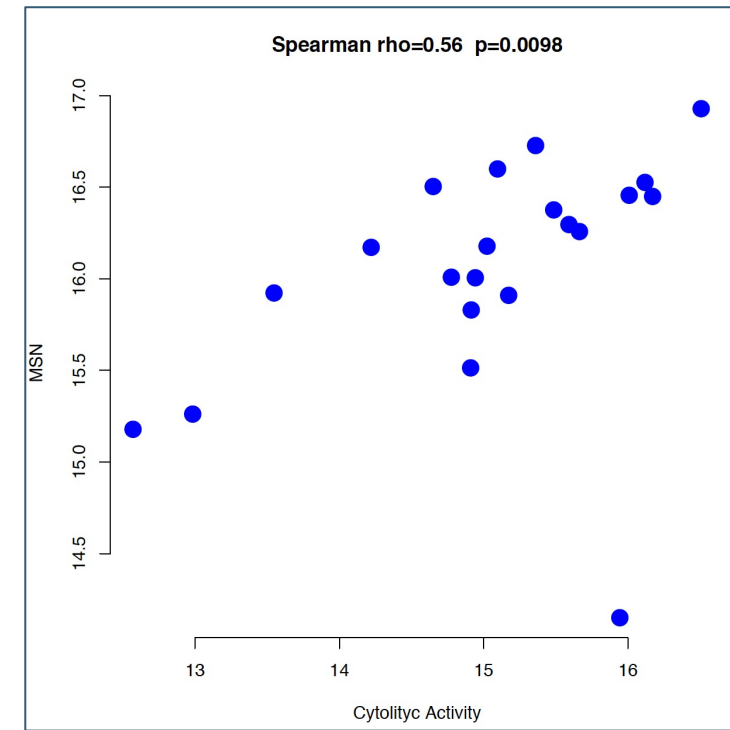
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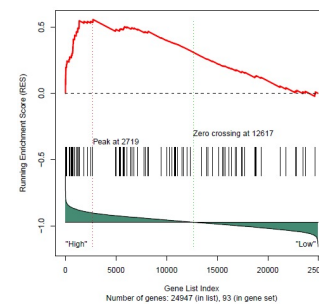
➤ RNA seq data (n=38)

- ✓ MSN inactivation is associated with lower cytolytic activity (Bulk++)
- ✓ GSEA of FcgR mediated phagocytosis and TCR pathway in MSN^{high} patients → Suggests downregulation of those pathways in case of MSN loss of function

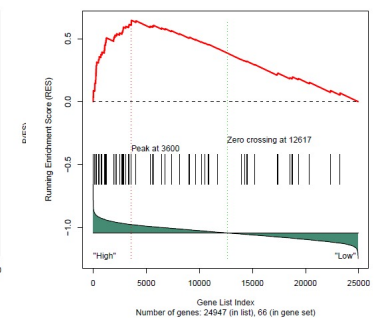
→ signature from tumor compartment or TME ?



Gene Set 124 : KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS



Gene Set 385 : PID_TCR_PATHWAY

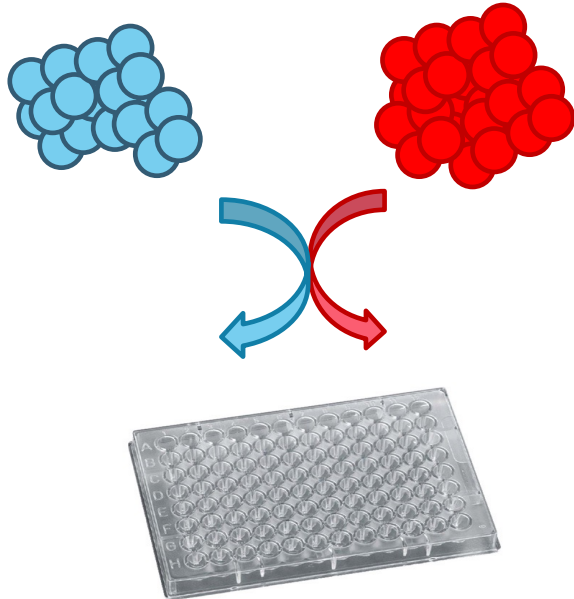


➤ Is MSN loss of function associated with ineffective immune response ?

ANALYZE THE RESISTANCE TO IMMUNE-MEDIATED KILLING

CD8+ T cells
+/- activated

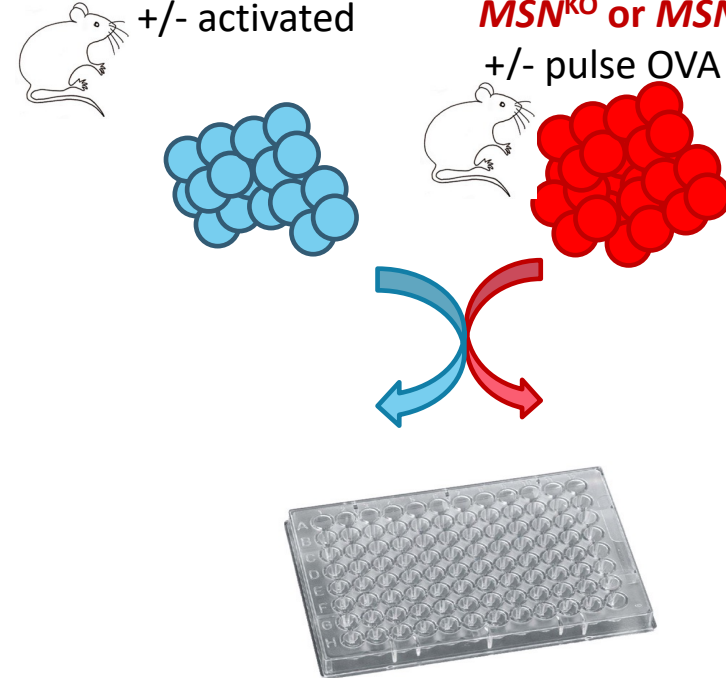
JURKAT red
MSN^{KO} or *MSN^{WT}*



Allogeneic immune response

OT-1 CD8+ T cells
+/- activated

EL4 red
MSN^{KO} or *MSN^{WT}*
+/- pulse OVA 1h



HLA restricted immune response

CYTOTOXICITY ASSAY

Incucyte

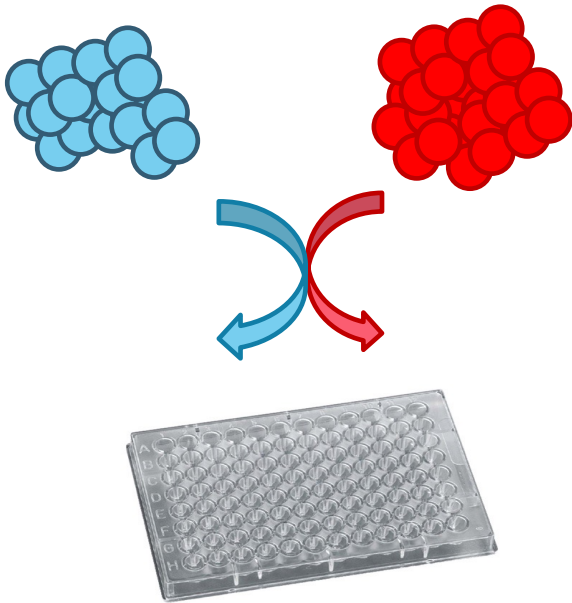
96 well plate

Analysis / 2h for 3 days

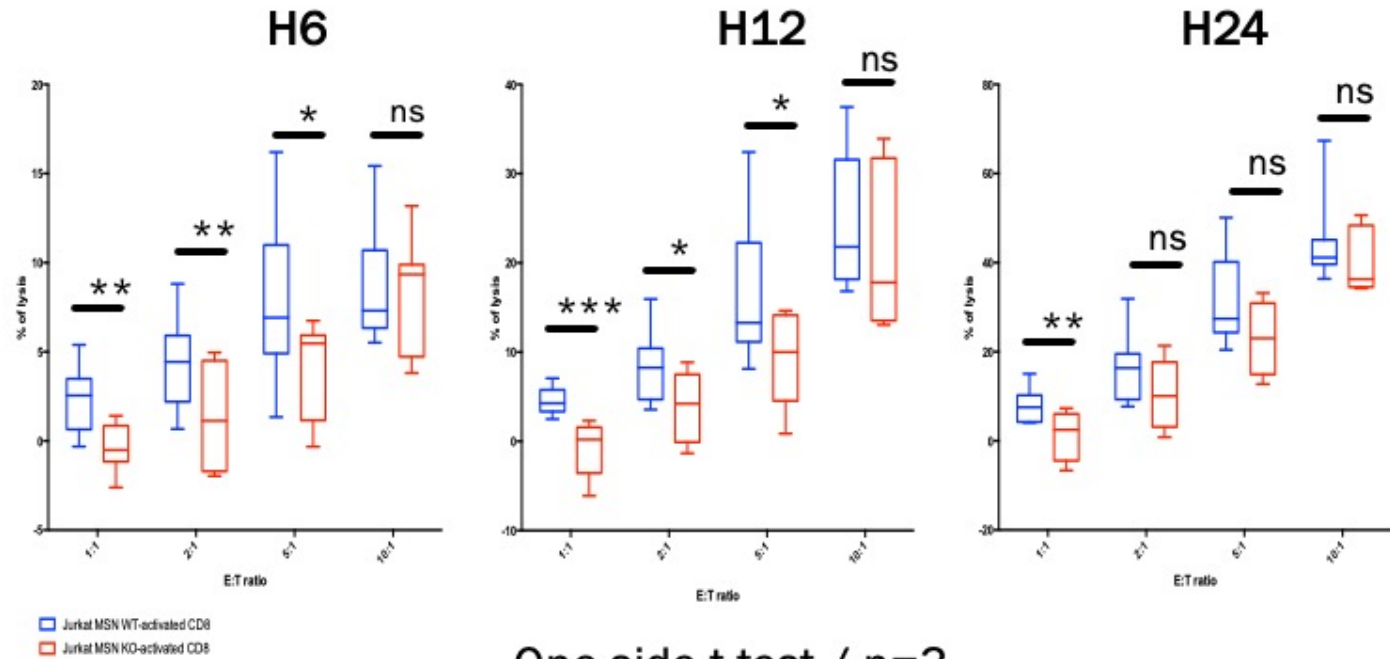
ANALYZE THE RESISTANCE TO IMMUNE-MEDIATED KILLING

CD8+ T cells
+/- activated

JURKAT red
 MSN^{KO} or MSN^{WT}



Allogeneic immune response

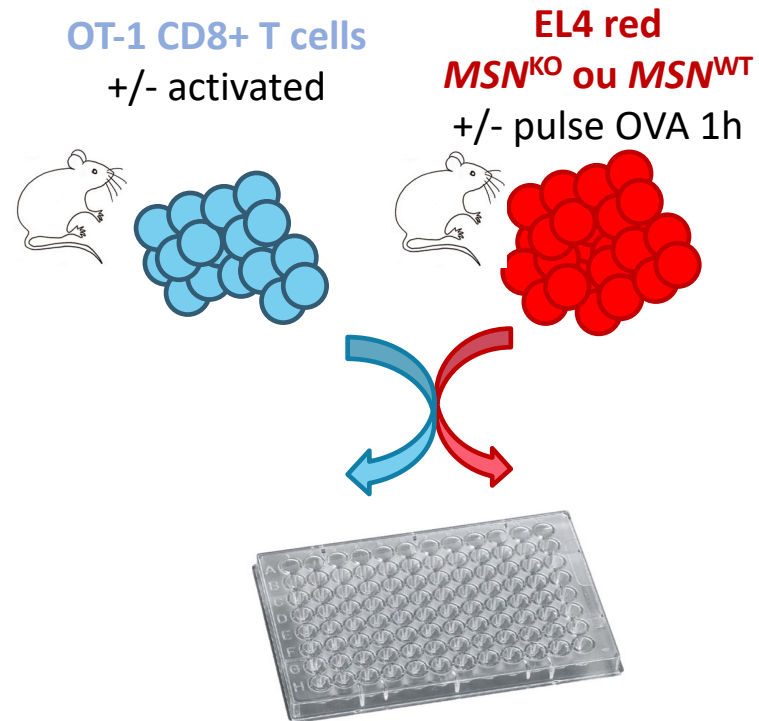


One-side t-test / n=3

- Trend for lower sensitivity to lysis in MSN^{KO} cells
- Very high % of lysis in this model

ANALYZE THE RESISTANCE TO IMMUNE-MEDIATED KILLING

Focus on the model based on AUTOLOGOUS RESPONSE
(MHC-restricted and antigen-specific)

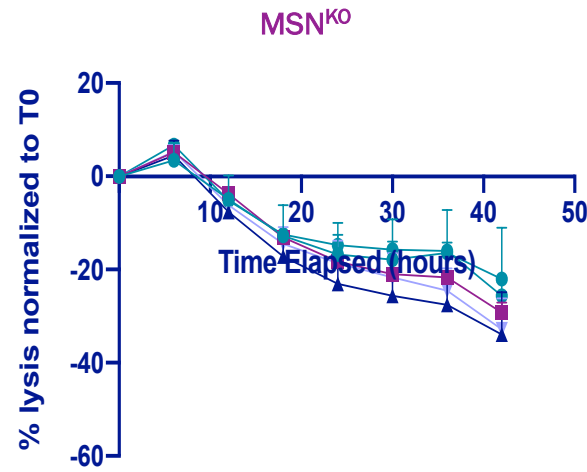
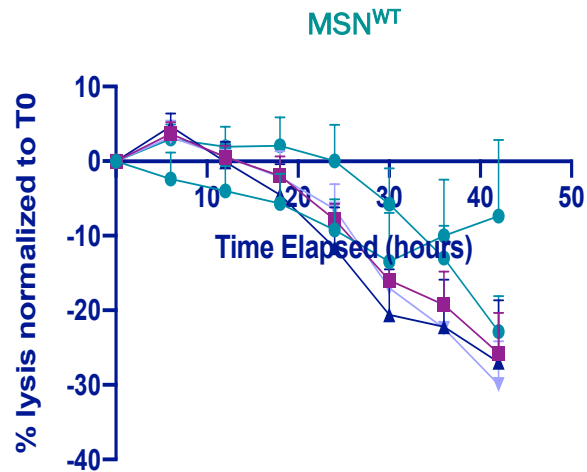


OT1 cells activation using total splenocytes (without irradiation) from WT mice as feeders + IL2

Day 5

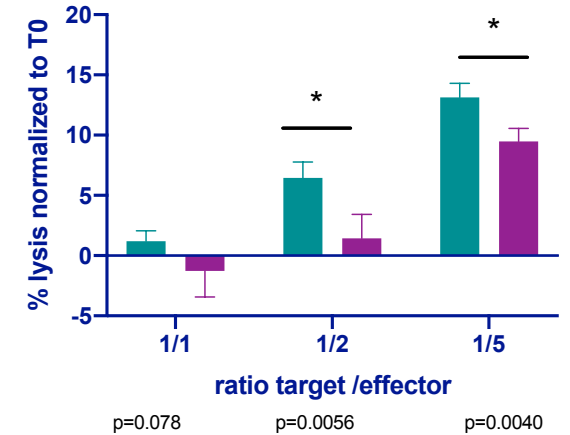
MSN^{WT}
MSN^{KO}

No pulse

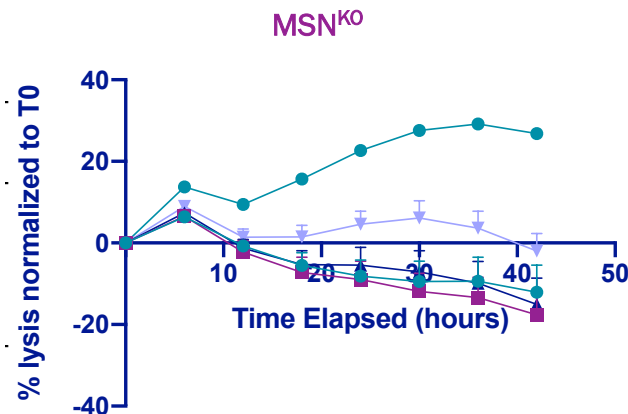
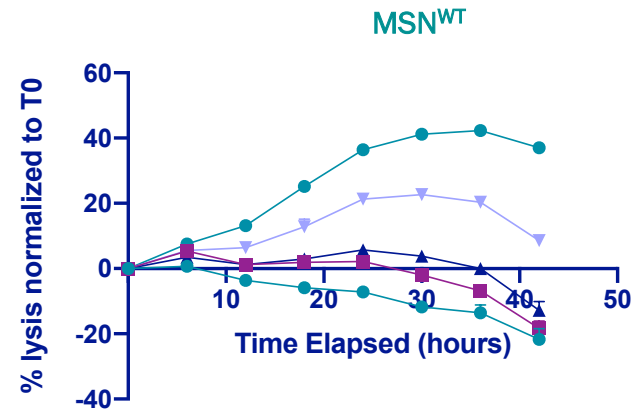


- EL4 B5 (-/-) no pulse + OT1 (1/0.1)
- EL4 B5 (-/-) no pulse + OT1 (1/0.5)
- EL4 B5 (-/-) no pulse + OT1 (1/1)
- EL4 B5 (-/-) no pulse + OT1 (1/2)
- EL4 B5 (-/-) no pulse + OT1 (1/5)

H12

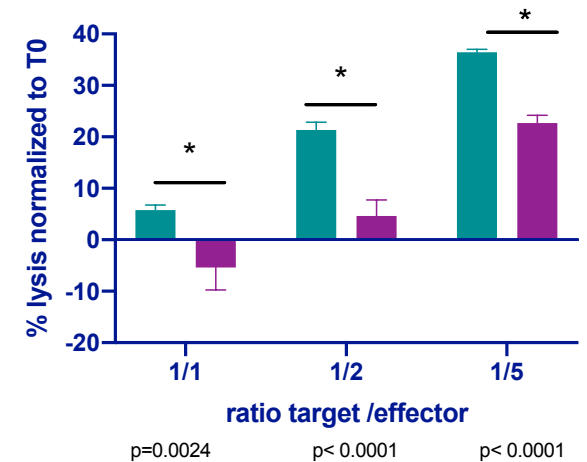


+ pulse OVA



- EL4 B5 (-/-) pulse OVA + OT1 (1/0.1)
- EL4 B5 (-/-) pulse OVA + OT1 (1/0.5)
- EL4 B5 (-/-) pulse OVA + OT1 (1/1)
- EL4 B5 (-/-) pulse OVA + OT1 (1/2)
- EL4 B5 (-/-) pulse OVA + OT1 (1/5)

H24



➤ No aspecific lysis at Day 5

➤ MSN^{KO} cells seems to be resistant to lysis at Day 5, consistent with lower activation of CD8+ T cells

EXPLORE THE MECHANISMS UNDERLYING RESISTANCE TO LYSIS

- In MSN^{KO} target cells:
 - CMH expression (Flow cytometry)
 - Immune synapse formation +/- stability (Amnis Imagestream)
 - PD-L1/PD-L2 expression (Flow cytometry)

CONCLUSION & PROSPECTS

- ❖ *MSN* inactivation may play a crucial role in NK/T lymphomagenesis, by disturbing antitumor immune response
- ❖ Identification of prognostic factors will be useful for patients' stratification in the era of personalized medicine
- ❖ Detection of new predictive biomarkers of response to antiPD1 therapy will allow to optimize use of immune checkpoint blockade in clinical practice

Characterization of immune escape mechanisms opens promising perspectives in the understanding of NKCTL pathogenesis and its treatment

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